ISOLATION AND CHARACTERIZATION OF BOVINE VASOACTIVE INTESTINAL PEPTIDE (VIP)

M. CARLQUIST, V. MUTT and H. JÖRNVALL
Departments of Biochemistry II and Chemistry 1, Karolinska Institutet, S-104 01 Stockholm, Sweden.

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1. Introduction

The vasoactive intestinal peptide (VIP) in the forms hitherto isolated is composed of 28 amino acid residues. In addition to the properties implied by its name, it possesses a broad spectrum of biological activities, although the physiological function is yet unknown. Originally isolated from upper intestinal tissue, VIP or material showing VIP-like immunoreactivity, has later been found in neural structures throughout the gastrointestinal tract, as well as in certain other areas of the peripheral nervous system, and in parts of the central nervous system. It has also been demonstrated in certain endocrine cells of the gastrointestinal tract [1]. The structure of the porcine peptide was first determined [2,3]. Chicken VIP was isolated later and its amino acid sequence was found to differ from porcine VIP in four residues, corresponding to an 86% identity [4].

This study reports an improved isolation procedure for VIP, the isolation of bovine VIP and the determination of its complete primary structure.

2. Experimental

2.1. *Isolation of bovine VIP*

The first meter of bovine upper intestine was removed from each animal within 30 min slaughter, boiled (10 min) and frozen. This material (100 kg) was minced and extracted at + 5°C with 200 l 0.5 M acetic acid. The suspension was filtered and peptides were adsorbed from the filtrate to 3.5 kg (wet wt) prewashed [5] alginic acid, (Landalgine P from Ed. Mendell Co., Carmel, NY). They were eluted with ice-cold 0.2 M HCl (30 l) and precipitated from the eluate with NaCl (320 g/ l) [5]. The precipitate (100 g wet wt) was collected by suction, and dissolved in 66% ethanol, at pH 7.2. The soluble peptides were resubmitted to the alginic acid and NaCl steps. The material was then extracted with methanol [5], the soluble peptides were precipitated with ether, and chromatographed on Sephadex G-25 fine (Pharmacia) in 0.2 M acetic acid. The fraction of VIP bioactivity was further chromatographed on a 2.5 × 16 cm column CM-cellulose CM 22 (Whatman). Elution was performed by a stepwise increase in ammonium bicarbonate from 0.02–0.06 M, 0.10 M and finally 0.20 M. The VIP fractions at 0.10 M were subjected to counter-current distribution in a 60-tube all glass apparatus (Spectrum Medical Industries) with the system 0.10 M ammonium bicarbonate:1-butanol (1:1, v/v) [2]. Final purification was carried out on a high-performance liquid chromatograph equipped with a μ-Bondapak C_{18} column (7.8 × 300 mm), a U6K injector, a M-6000 A pump and a 450 UV-detector (Waters Associates). The flow rate was 2.0 ml/min and the solvent system consisted of 0.005 M ammonium acetate in 36% ethanol (pH 4.6) as measured with a glass electrode after adjustment with acetic acid. Purity was confirmed by isotachophoresis [6] and by increasing secretin-like activity [7].

2.2. *Structural analysis*

Digestion of bovine VIP (134 μg) with TPCK-treated trypsin (5 μg) in 0.1 M ammonium bicarbonate (50 μl) was performed at 37°C for 4 h. Fragments were separated by high-voltage paper electrophoresis

at pH 6.5 [8], and eluted with water. Total compositions were determined on a Beckman 121 M amino acid analyser, after hydrolysis at 110°C for 24 h in evacuated tubes with 6 M HCl containing 0.5% phenol. N-terminal amino acids were analyzed by the dansyl method and manual sequence analysis was performed with the dansyl-Edman method [8,9]. Liquid phase sequencer degradation was carried out in a Beckman 890 C instrument, using the 0.1 M quadrol peptide program and double identifications of residues liberated [10]. PTH-amino acids were analyzed by high-performance liquid chromatography [11] and by thin-layer chromatography [12]. The C-terminal amidated residue was determined according to the method using chymotrypsin and identification as dansyl amino acid amide [13].

3. Results and discussion

3.1. *Isolation of bovine VIP*

Bovine VIP was obtained in 4 times higher yield than VIP purified from pig. Although from a different species the increased amount is probably mainly due to the present modifications in the purification method. The Sephadex G-25 fractionation was now carried out after, instead of before, the methanol extraction [2], thus producing a salt-free material for the subsequent step. The CM-cellulose column was now equlibrated with 0.02 M ammonium bicarbonate, at pH 8.0. The active material, 600 mg from the Sephadex G-25 fractionation, was dissolved in a small volume of water and adjusted to pH 8.0 with 0.02 M ammonia, the sample solution thereby reaching a volume of ~25 ml. A small precipitate was removed by centrifugation and the supernatant was applied to the column, followed by stepwise elution with increasing concentration of ammonium bicarbonate (fig.1.). The fractions that showed secretin-like activity in the cat were combined and lyophilized. This material (25 mg) was subjected to counter-current distribution, as described in section 2, and yielded 4 mg. Final purification was obtained by high-performance liquid chromatography as illustrated in fig.2.

3.2. *Sequence determination of bovine VIP*

Bovine VIP (134 μg) was digested with trypsin and the fragment mixture obtained was separated into 6

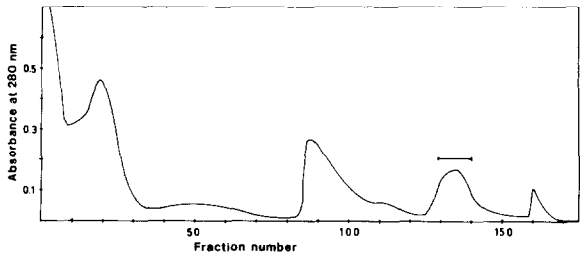

Fig.1. Chromatography on CM-cellulose (2.5 × 16 cm) of peptides (600 mg) from the Sephadex G-25 fine step. Elution in 10 ml fractions with NH_4HCO_3, 0.02 M (fractions 1–77), 0.06 M (78–121), 0.10 M (122–149) and 0.20 M (150–170). The VIP fraction is indicated by the bar.

peptides and 1 free amino acid (lysine) by high-voltage paper electrophoresis. The amino acid compositions and the N-terminal residues of all tryptic fragments as well as the intact peptide are shown in table 1. The complete sequence of the peptide is given in fig.3, together with the methods of residue identifications. The amount of bovine VIP used in the liquid-phase sequencer analysis was 300 μg (corresponding to maximally 100 nmol), and 2 extra cycles were per-

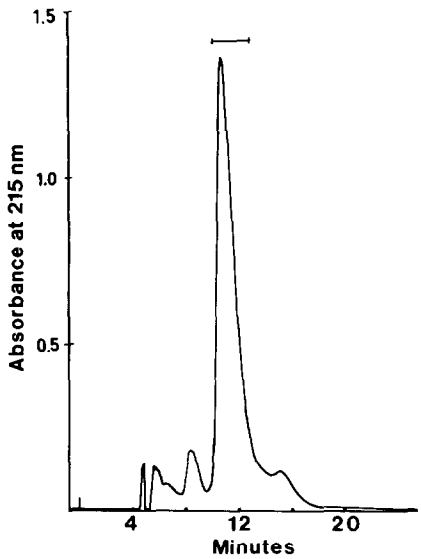

Fig.2. High-performance liquid chromatography of 500 μg bovine VIP-active material from the counter-current distribution. Column: 7.8 × 300 mm (μ-Bondapak C_{18}). Flow rate 2.0 ml/min. Solvent: 0.005 M CH_3COONH_4 in 36% ethanol (pH 4.6). The VIP fraction is indicated by the bar.

Table 1
Data for bovine VIP and its tryptic peptides

Composition	Acid hydrolysis	Sum of sequence	Tryptic peptides T_1	T_2	T_3	T_4	T_5	T_6	T_7
Asx	5.2 (5)	5	2.8 (3)	–	–	–	–	–	2.1 (2)
Thr	2.0 (2)	2	1.9 (2)	–	–	–	–	–	–
Ser	2.1 (2)	2	1.2 (1)	–	–	–	–	–	1.1 (1)
Glx	1.0 (1)	1	–	–	–	1.0 (1)	1.0 (1)	–	–
Ala	2.0 (2)	2	1.1 (1)	–	–	1.1 (1)	1.0 (1)	–	–
Val	2.0 (2)	2	1.0 (1)	–	–	0.9 (1)	1.0 (1)	–	–
Met	1.0 (1)	1	–	–	–	0.9 (1)	1.0 (1)	–	–
Ile	1.0 (1)	1	–	–	–	–	–	–	1.0 (1)
Leu	2.9 (3)	3	–	1.0 (1)	0.8 (1)	–	–	–	1.8 (2)
Tyr	1.8 (2)	2	0.9 (1)	–	–	–	–	–	1.0 (1)
Phe	1.0 (1)	1	0.9 (1)	–	–	–	–	–	–
His	1.0 (1)	1	1.2 (1)	–	–	–	–	–	–
Lys	3.0 (3)	3	–	–	1.2 (1)	2.0 (2)	1.0 (1)	(1)	–
Arg	2.2 (2)	2	0.9 (1)	1.0 (1)	0.9 (1)	–	–	–	–
Total	28	28	12	2	3	6	5	1	7
N-terminus	His		His	Leu	Leu	Lys	Gln	Lys	Tyr
Recovery (%)			30	19	12	9	27		18
Mobility			0.16	– 0.58	– 0.85	– 0.64	– 0.36	– 0.91	– 0.25

Values are molar ratios without corrections for destruction, incomplete hydrolysis or impurity. Cys, Gly, Pro and Trp are absent from all peptides. Electrophoretic mobility is given relative to aspartic acid, at pH 6.5.

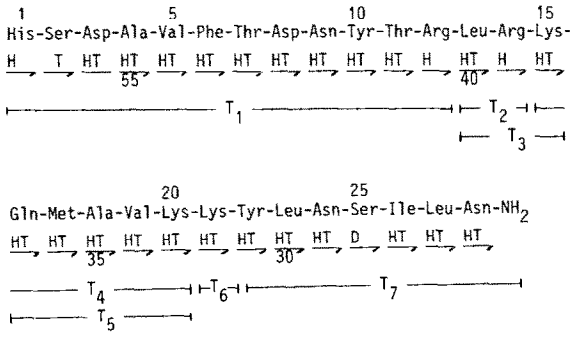

Fig.3. The amino acid sequence of bovine VIP. Sequencer analysis is shown by (——→); the tryptic peptides by T_1–T_7, residue identifications by H (high-performance liquid chromatography), T (thin-layer chromatography) or D (dansyl-Edman method), and nmol recovered by figures below the arrows. Repetitive yield calculated on Ala_{4-18} is 97% and on Leu_{13-23} is 97%.

formed after step 28, but as expected no further residues could be identified. Residue 25 was confirmed as serine by analysis of the tryptic fragment T_7, using manual degradation with the dansyl-Edman method. The total compositions, electrophoretic mobilities and N-terminal residues of all tryptic fragments are in perfect agreement with the sequence determined for the whole structure. In addition the sum of amino acids from the sequencer analysis fits exactly with that from the acid hydrolysis (table 1).

3.3. *Variations among related peptide hormones*

With the present determination, VIP structures have been characterized, those from pig [3] and chicken [4] in addition to the bovine peptide now analyzed. A comparison of these shows that bovine and porcine VIP are identical. However, amino acid sequence differences do occur between many other

homologous peptide hormones from these two species. This has been shown for ACTH, calcitonin, gastrin, PTH and insulin [14,15]. In the case of glucagon however the bovine and porcine hormones are identical, thus resembling the situation for VIP. Indeed no differences have been observed in any of the mammalian glucagons [16]. The present finding of identical bovine and porcine VIP structures may suggest that VIP, like glucagon, is strongly conserved during evolution, and that large sections of the VIP peptide chain are essential for the biological activity.

Acknowledgements

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