Valosin: isolation and characterization of a novel peptide from porcine intestine

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The isolation and primary structure of a novel gastrointestinal peptide, designated valosin, is described. The peptide was purified from porcine upper gut extracts using an HPLC and N-terminal sequence screening strategy which depends on chromatographic and structural characteristics as isolation criterion. The amino acid sequence of this peptide consists of 25 amino acid residues:

V Q Y P V E H P D K F L K F G M T P S K G V L F Y.

It does not show structural homology to known protein or nucleotide sequences. According to preliminary experiments, valosin possesses biological effects on the gastrointestinal tract of the dog.

Valosin *Gastrointestinal peptide* *Isolation* *Porcine gut extract* *HPLC-sequence screening* *Primary structure*

1. INTRODUCTION

A number of gastrointestinal peptides, for instance PHI [1], PYY [2] and galanin [3], have been isolated from porcine intestinal extracts using a chemical assay based on the detection of the C-terminal amide structure without having any information on a biological activity [4]. Subsequent studies revealed that these peptides have biological activities and may exert endocrine or neurocrine regulatory functions in the gut and brain of different species. Since not all biologically active peptides contain a C-terminal amide, e.g. glucagon, motilin, GIP, somatostatin and neurotensin, we used a screening strategy which is dependent on chromatographic behaviour, UV-absorbance characteristics and N-terminal sequence determination to isolate and characterize novel peptides from porcine intestinal tissue extracts. While investigating side-fractions arising from the purification scheme for PHI and secretin, we identified a novel peptide with an HPLC retention time different from the known gastrointestinal peptides.

We report here the isolation and structural characterization of this novel gastrointestinal peptide which we have designated 'valosin' from N-terminal valine and C-terminal tyrosine. This peptide was formerly referred to as 'peptide VQY' in a preliminary account of this study [5].

2. MATERIALS AND METHODS

2.1. *Isolation procedures*

As starting material a side-fraction of the secretin purification scheme [6] was used. Briefly, upper porcine intestine (4.0 kg) was boiled in water for 10 min, frozen, minced and extracted with 0.5 M acetic acid. Peptides were further purified

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by adsorption to alginic acid, extraction with 66% ethanol, gel filtration on Sephadex G-25 and extraction with methanol. The methanol-soluble fraction, also containing secretin, PHI and PYY, was chromatographed on CM-cellulose in 12.5 mM Na-phosphate, pH 8.0. All fractions eluting before secretin were pooled and used as starting material. This was subjected to another CM-cellulose column and eluted with 0.02 M NH_4HCO_3 (pH 6.5) followed by 0.2 M, pH 8.0. All resulting fractions (I–IX) were characterized by HPLC on a reversed-phase C-18 column (Shandon ODS Hypersil, 5 μm or Vydac RP-201 widepore, 300 Å, 10 μm) using a Waters HPLC system equipped with a dual-channel UV detector (M440) for measurement at 214 and 280 nm. The chromatographic conditions are described in the legends to figs 2 and 3. Unknown peptide peaks were rechromatographed and characterized by HPLC retention time compared to mixtures of known gastrointestinal peptides. For further identification the UV absorbance was measured at 214 nm (sensitivity, 0.1 units) and 280 nm (0.01). The peaks were integrated and the ratio R of the areas was calculated as $R = F_{(214/0.1)}/F_{(280/0.01)}$. This ratio was considered to represent a characteristic value for each peptide reflecting the relative content of amino acids with high absorption at 280 nm, i.e. tryptophan and tyrosine.

2.2. *Structural analysis*

Amino acid analysis was performed with a Durrum D-500 automatic amino acid analyzer after hydrolysis of the sample with 0.5 ml of 5.7 N HCl containing 0.5% phenol in evacuated tubes at 110°C for 24 h. For sequence determination the peptide was cleaved by TPCK-trypsin in 0.1 M NH_4HCO_3 (pH 8.2) for 6 h at 37°C, the tryptic fragments were isolated by HPLC. The amino acid sequence was determined by manual DABITC-Edman degradation (dimethylaminoazobenzene isothiocyanate method) [7] for N-terminal sequence screening and for sequencing of the tryptic peptides. Liquid-phase sequencer degradation on a Beckman model 890 with spinning-cup modification [8] and gas-phase sequencing on an Applied Biosystems model 470A [9], both in the presence of polybrene [10], were used for the intact peptide and the tryptic peptide T4. The phenylthiohydantoin derivatives were identified by HPLC on a reversed-phase microbore column [11]. The obtained primary structures were screened for sequence homology against the Dayhoff's Protein Segment Dictionary [12] and against the protein and nucleotide sequence data bank constructed by Doolittle et al. [13].

3. RESULTS

3.1. *Isolation of the peptide*

Fig.1 shows the ion-exchange chromatography on CM-cellulose in 0.02 M and subsequently 0.2 M ammonium bicarbonate of the 'presecretin' fraction. Fractions 1–IX were collected and further separated by reversed-phase HPLC. The peptide peaks were characterized by their HPLC retention times in a trifluoroacetic acid (TFA)/acetonitrile system (see fig.2 legend) and by the ratio of UV absorbance at 214 and 280 nm. Using this strategy combined with a screening N-terminal sequence determination of the first 4–6 residues by manual DABITC-Edman degradation, a novel peptide could be identified in fraction VI of the CM-cellulose chromatography. Fig.2 shows the HPLC elution profile of this fraction. Furthermore, chromatographic and sequence analysis unequivocally identified in fraction VI some other peptide peaks additional to the well-characterized peptides PYY, secretin and PHI, as marked in fig.2. Rechromatography of the peak, preliminarily designated VQY on the basis of the first 3

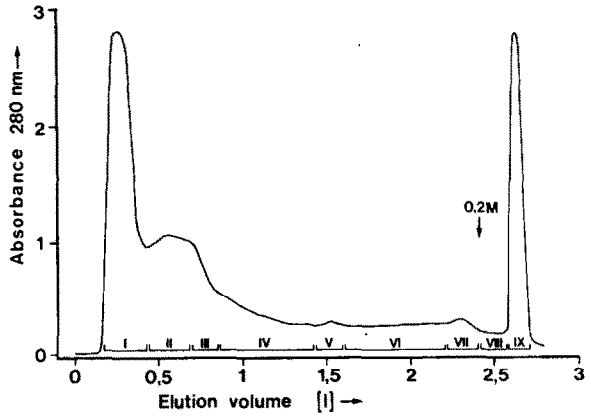

Fig.1. Cation-exchange chromatography of the starting material (2.0 g) on a CM-cellulose column (5 × 15 cm) in 0.02 M/0.2 M NH_4HCO_3. Flow rate, 10 ml/min; fractions I–IX were further characterized.

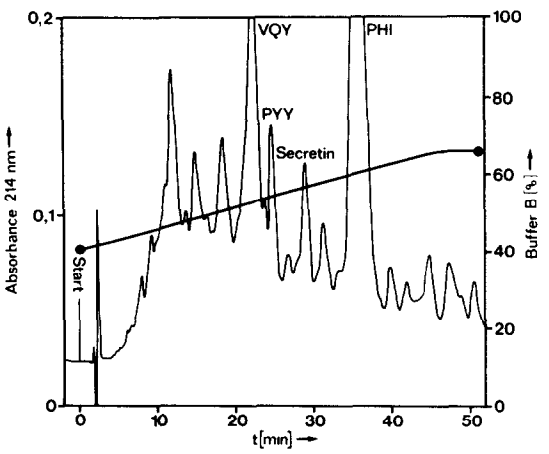

Fig.2. HPLC elution profile of fraction VI from the CM-cellulose chromatography (fig.1). 0.6 mg was applied to a Shandon ODS Hypersil reversed-phase C-18 column (5 μm, 0.46 × 25 cm) and eluted at 40°C using a linear gradient from 45 to 65% solvent B. Flow rate, 1.5 ml/min; solvent A, 0.1% TFA; solvent B, 0.12% TFA in 70% acetonitrile. PYY, secretin and PHI were identified by retention-time analysis and confirmed by N-terminal sequence determination, the peak marked VQY contained the peptide valosin. UV absorbance at 214 and 280 nm (not shown) was measured.

residues, on a reversed-phase C-18 wide-pore column, using a shallow gradient in the TFA/acetonitrile system, resulted in isolation of the peptide in essentially pure form as shown by the elution profile in fig.3.

3.2. *Structural analysis*

The amino acid analysis (table 1) revealed that the peptide consists of 25 amino acid residues. Based on its N- and C-terminal amino acids (valine and tyrosine, respectively) the name valosin is proposed for this peptide. The tryptic fragments T1–T4 were isolated by HPLC using the same system as described in the legend to fig.2, their amino acid compositions being listed in table 1. Using manual DABITC-Edman degradation, the primary structures of the peptides T1, T2 and T3 were completely established and the intact peptide could be sequenced up to position 18 by liquid-phase sequencing. Due to its substantial hydrophobicity, peptide T4 was washed out after step 2 of sequencing with both methods. However, using the gas-phase sequencing technique, the

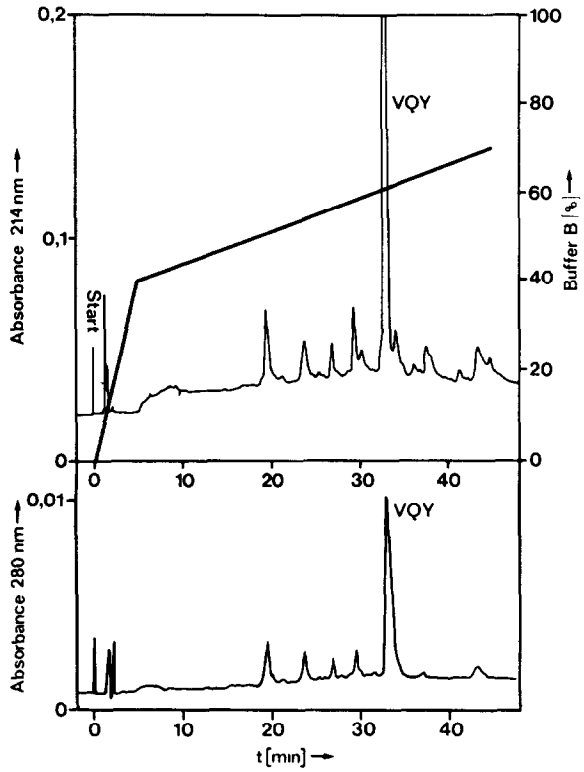

Fig.3. HPLC rechromatography of the peak containing peptide valosin (VQY) on a Vydac RP-201 C-18 wide-pore column (300 Å, 10 μm) using the same solvent system as described in fig.1. Peptide valosin eluted in pure form as indicated by the absorbance profile read at 214 and 280 nm.

Table 1

Amino acid composition of peptide valosin and its tryptic fragments T1, T2, T3 and T4

	Valosin	T1	T2	T3	T4
Asx	1.2 (1)	1.1 (1)			
Thr	1.0 (1)			0.9 (1)	
Ser	1.0 (1)			1.2 (1)	
Glx	2.3 (2)	2.1 (2)			
Pro	3.1 (3)	2.1 (2)		0.9 (1)	
Gly	2.1 (2)			1.3 (1)	1.2 (1)
Val	2.8 (3)	1.8 (2)			0.9 (1)
Met	0.9 (1)			0.8 (1)	
Leu	2.1 (2)		1.2 (1)		0.8 (1)
Tyr	1.9 (2)	1.0 (1)			1.0 (1)
Phe	2.9 (3)		0.8 (1)	0.9 (1)	1.0 (1)
His	1.0 (1)	0.8 (1)			
Lys	2.9 (3)	1.1 (1)	1.0 (1)	1.1 (1)	
Sum	25	10	3	7	5

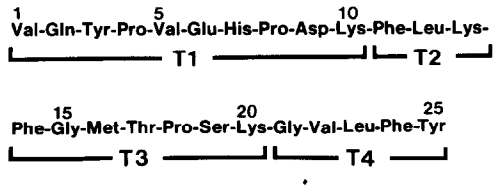

Fig.4. The complete primary structure of peptide valosin (VQY) and its tryptic fragments T1–T4.

complete amino acid sequence of peptide T4 and of intact valosin was elucidated as shown in fig.4, using 1.0 and 0.5 nmol, respectively. The C-terminus of both peptides was not amidated.

4. DISCUSSION

Using an HPLC- and N-terminal sequence screening strategy, a peptide with a novel primary structure was isolated from highly purified peptide fractions derived from porcine intestinal extracts. The amino acid sequence was searched for structural homology against Dayhoff's Protein Segment Dictionary [12] and against the protein and nucleotide sequence data bank of Doolittle et al. [13]: no convincing homology was found to any known peptide or protein sequence except from coincidentally identical residues. It appears, therefore, that valosin is a novel peptide and may be a member of an as yet unidentified family of gastrointestinal peptides.

In preliminary screening experiments looking for biological effects in dogs equipped either with pancreatic fistulas or with implanted electrodes along the small bowel, the peptide valosin seemed to exert a dose-dependent CCK- or bombesin-like stimulatory action on exocrine pancreatic secretion, whereas the appearance of the slow migrating myoelectric complexes in different parts of the intestine was delayed [5]. Despite the preliminary character of these biological studies, the results may point to a regulatory function for valosin in the gastrointestinal tract.

As yet, however, the possibility cannot be excluded that the peptide valosin will be identified as a fragment of a hitherto unknown protein in the gut, since other protein fragments, e.g. from α-chain of hemoglobin, have been shown to exhibit certain biological actions [14,15].

Immunocytochemical studies on the distribution within the gut and molecular cloning of the precursor molecule of peptide valosin should help to clarify its role in the gastrointestinal tract.

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