ACTIVATION PEPTIDE OF HUMAN TRYPSINOGEN 2

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1. Introduction

The sequence of the activation peptide liberated during the conversion of trypsinogen into active trypsin has been determined in several mammalian species [1–12] and in two fishes, the dogfish and the lungfish [13–15]. It is an N-terminal peptide whose general structure is X–Asp–Asp–Asp–Asp–Lys in all cases except the lungfish whose sequence is Phe–Pro–Ile–Glu–Glu–Asp–Lys. The length of the peptide varies from 6 to 8 amino acid residues according to the species.

The determination of the different sequences has permitted to establish phylogenetic relationship between species on purely biochemical date. Moreover, the physiological importance of this peptide has been demonstrated by Abita et al. [16] who observed on inhibitory effect on gastric secretion. These observations underline the interest of the characterization of the activation peptides of human trypsinogens.

In this work, we report the isolation and sequence of the activation peptide of human trypsinogen 2, the most anionic trypsinogen isolated from human pancreatic juice [17].

2. Materials and methods

2.1. *Materials*

Human trypsinogen 2 was isolated from pancreatic juice according to the method described previously [18].

Trypsin inhibitor-Sepharose was prepared by activating Sepharose 4B with cyanogen bromide and coupling activated Sepharose with kallikreintrypsin-inhibitor® (Bayer AG) according to the procedure of Cuatrecasas et al. [19].

2.2. *Activation of trypsinogen*

12 mg of trypsinogen were dissolved in 4 ml of activation buffer (5 mM Tris–HCl buffer containing 20 mM $CaCl_2$ and 40 mM NaCl) at 0°C. When the pH was adjusted at 7.8 generally trypsinogen activated spontaneously. When the activation was too slow, 120 μg of pure porcine enterokinase (a gift of Dr S. Maroux) were added to the activation mixture. After 2 h, the activity reached a maximal constant value and the activation was stopped by inhibition of trypsin with kallikrein trypsin inhibitor coupled to Sepharose.

2.3. *Isolation of the activation peptide*

The activation mixture was submitted to an affinity chromatography on a column of trypsin inhibitor-Sepharose (0.9 × 10 cm) equilibrated at pH 7.8 with the activation buffer containing 0.2 M NaCl. The peptides eluted with the breakthrough peak were lyophilised and filtered through a column of Sephadex G-25 (fine) (0.9 × 200 cm) equilibrated with 10% acetic acid. The activation peptide was finally purified by high voltage electrophoresis at pH 5.3 on Whatman 3 MM at 3000 V for 35 min. The peptide was eluted from paper by the mixture pyridine-water (1/1 by vol.) and analyzed.

2.4. *Peptide analysis*

Peptides were detected by reaction of aliquots

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Table 1
Analysis of the activation peptide of hyman trypsinogen 2. The underlined numbers show the residues released by one cycle of subtractive Edman degradation

	Lys	Asp	Pro	Ala	Phe
Amino acid composition	1.04	4.02	1.12	0.78	0.86
Edman degradation					
– step 1	0.71	4.10	1.28	0.24	0.90
– step 2	0.58	4.00	0.55	0.28	0.84

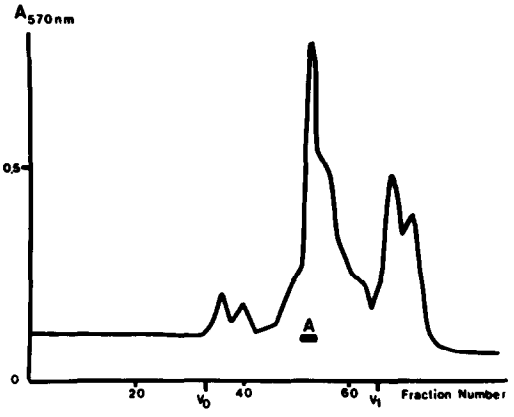

Fig.1. Gel filtration on Sephadex G-25 of peptides liberated by activation of trypsinogen 2. The column (0.9 × 200 cm) was equilibrated with 10% acetic acid. The effluent was collected in 2.2 ml fraction. Peptides were located by ninhydrin analysis after alkaline hydrolysis.

of column effluents with ninhydrin following alkaline hydrolysis. Absorbance was read at 570 nm. Amino acid compositions of peptides were determined using a Jeol Analyzer 5 AH, after 18 h hydrolysis at 110°C with 6 N HCl in evacuated sealed tubes.

N-terminal residues of peptides were identified by dansylation according to Hartley [20] or by Edman degradation using a subtractive procedure [21].

3. Results and discussion

Filtration of peptides on Sephadex G-25 is represented in fig.1. Fractions corresponding to the major peak of absorption at 570 nm (pool A) were pooled, concentrated and submitted to paper electrophoresis. The peptide map is reproduced in fig.2. After analysis of the different spots, the activation peptide (hatched spot) was found in the anodic part as expected, due to the presence of several aspartic acid residues. Table 1 gives the amino acid composition and two steps of Edman degradation of this peptide. The N-terminal

Table 2
Sequences of activation peptides of trypsinogens of different species

	Dogfish	Ala–Pro–Asp–Asp–Asp–Asp–Lys	Bradshaw et al. [13]
	Lungfish	Phe–Pro–Ile –Glu–Glu–Asp–Lys	Hermodson et al. [14]
	Horse	Ser–Ser–Thr–Asp–Asp–Asp–Asp–Lys	Harris and Hofmann [8]
	Elephant seal	Phe–Pro–Thr–Asp–Asp–Asp–Asp–Lys	Bricteux et al. [10]
	Lesser rorqual	Phe–Pro–Ile –Asp–Asp–Asp–Asp–Lys	Bricteux et al. [11]
	Dromedary	Val–Pro–Ile –Asp–Asp–Asp–Asp–Lys	Bricteux et al. [9]
	Red deer	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys	Bricteux et al. [7]
		Val–Asp–Asp–Asp–Asp–Lys	
	Roe deer	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys	Bricteux [6]
		Val–Asp–Asp–Asp–Asp–Lys	
	Sheep	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys	Bricteux et al. [4]
		Val–Asp–Asp–Asp–Asp–Lys	
	Goat	Phe–Pro–Val–Asp–Asp–Asp–Asp–Lys	Bricteux et al. [5]
		Val–Asp–Asp–Asp–Asp–Lys	
Bovine	cationic trypsinogen	Val–Asp–Asp–Asp–Asp–Lys	Davie and Neurath [1]
	anionic trypsinogen	Phe–Pro–Ser–Asp–Asp–Asp–Asp–Lys	Louvard and Puigserver [12]
		Ser–Asp–Asp–Asp–Asp–Lys	
Porcine	cationic trypsinogen	Phe–Pro–Thr–Asp–Asp–Asp–Asp–Lys	Charles et al. [2]
	anionic trypsinogen	Phe–Pro–Thr–Asp–Asp–Asp–Asp–Lys	Louvard and Puigserver [12]

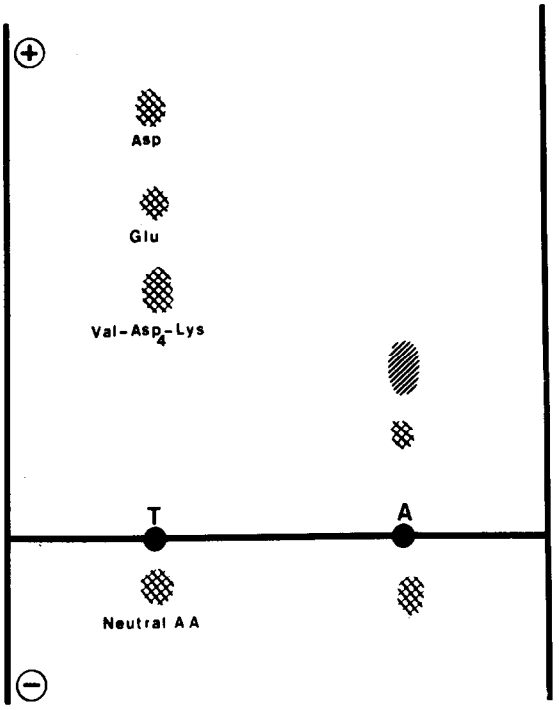

Fig.2. Reproduction of the high-voltage paper electrophoresis of pool A eluted from Sephadex G-25. On the left, a mixture of standard amino acids containing the activation peptide of bovine trypsinogen, Val–$(Asp)_4$–Lys, is given as reference.

residue of alanine was characterized by Edman degradation and by dansylation. These results lead to the conclusion that the activation peptide of human trypsinogen 2 is an octapeptide with the sequence:

Ala–Pro–Phe–Asp–Asp–Asp–Asp–Lys

Like all mammalian trypsinogens except the bovine cationic one (table 2) the activation peptide of human trypsinogen 2 is an octapeptide containing the constant sequence Asp–Asp–Asp–Asp–Lys. It is interesting to note the persistance of proline in position 2 as in each mammalian octapeptide except for that of the horse. The occurrence of phenylalanine in position 3 of the peptide from human trypsinogen 2 makes it unlike any of the previously described octapeptides where phenylalanine, if present, occupies position 1. This replacement and the substitution of the N-terminal residue of phenylalanine by a residue of alanine which requires two base mutations raises the question of the hypothetical ancestral sequence of the activation peptides. The sequence of the activation peptide of human trypsinogen 2 would favor the ancestral sequence Ala–Pro–Ile–$(Asp)_4$–Lys one of the two ancestral sequences proposed by Reeck and Neurath [15].

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