PORCINE TRYPSIN: SEPARATION OF THE TWO POLYPEPTIDE CHAINS OF THE α-FORM AND PARTIAL AMINO ACID SEQUENCE*

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1. Introduction

We have recently described the separation and characterisation of different active forms of porcine trypsin [1]. One of these forms, β-trypsin, was shown to have a single polypeptide chain and another form α-trypsin was found to differ from β-trypsin in so far as it has an interchain split between a lysine and a serine residue and hence is composed of two polypeptide chains linked by disulphide bridges.

In the present work we wish to describe the chromatographic separation of the two polypeptide chains of α-trypsin and the determination of part of their amino acid sequences.

2. Methods

2.1. *Enzymes*

α-Trypsin and β-trypsin were prepared from a commercial preparation as described previously [1].

2.2. *Reduction and alkylation of disulphide bridges*

α- and β-Trypsins previously inactivated by treatment with diisopropylphosphofluoridate [1] were reduced with mercaptoethanol and either carboxymethylated with iodoacetic acid [2] or aminoethylated with ethylenimine [3]. The resulting proteins were separated from low molecular weight contaminants on Sephadex G-75 as described below.

2.3. *Separation of the two chains of α-trypsin*

This was effected on a column of Sephadex G-75 (110 × 5 cm) equilibrated with 50% acetic acid. The reaction mixture obtained after alkylation was applied directly to this column. The optical density of fractions at 280 nm was measured. Fractions containing proteinaceous material were pooled, concentrated by rotatory evaporation under vacuum at 45°, diluted to twice their volume with distilled water and freeze dried. Alkylated β-trypsin was separated from by-products of the alkylation reaction under similar conditions.

2.4. *Polyacrylamide electrophoresis in the presence of sodium dodecylsulphate*

This was performed according to Weber and Osborne [4]. The gels were stained and destained as described earlier [1].

2.5. *Amino acid analyses*

Proteins were analysed for their contents of amino acids as in [1]. Tryptophan was measured according to Liu and Chang [5].

2.6. *Automatic amino acid sequence analysis*

This was performed according to the general method of Edman and Begg [6] with the aid of a Beckman Sequencer (Model 890 C) following the fast protein, quadrol double cleavage program (Beckman program

* This paper is dedicated to Professor E. Lederer on the occasion of his 65th birthday.

no. 072172 c). Phenylthiohydantoin amino acids were identified by gas chromatography before and after silylation [7], by thin-layer chromatography on silica gel plates [8] and in the case of arginine and histine by specific staining reactions [9, 10].

All sequences were determined at least twice.

3. Results

3.1. *Separation of the two polypeptide chains of α-trypsin*

As shown in fig. 1 the two chains of α-trypsin were separated by chromatography of Sephadex G-75 in 50% acetic acid after prior reduction and carboxymethylation.

Gel electrophoresis in the presence of sodium dodecyl sulphate indicated that the two fragments obtained were homogeneous (fig. 2) and corresponded to peptides with molecular weights of approx. 13,000 and 11,000.

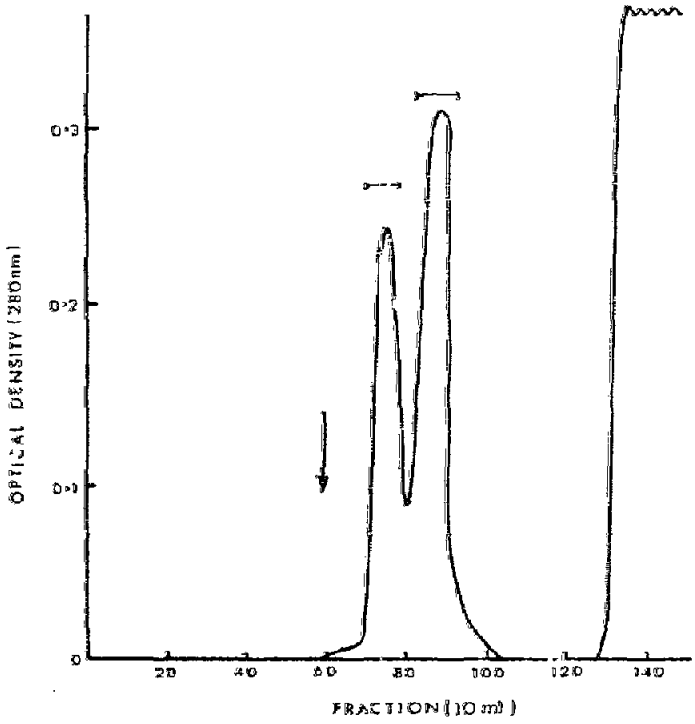

Fig. 1. Separation of the two chains of α-trypsin on Sephadex G-75. The enzyme (60 mg) was inactivated with diisopropylphosphofluoridate, carboxymethylated and applied to the column as described in Methods. The bars indicate the fractions which were pooled and the arrow the position of elution of carboxymethylated β-trypsin from the same column. Urea and salts eluted at 1300 ml. Fractions 71–78 gave 29.0 mg of material and 82–91 27.0 mg material after lyophilization.

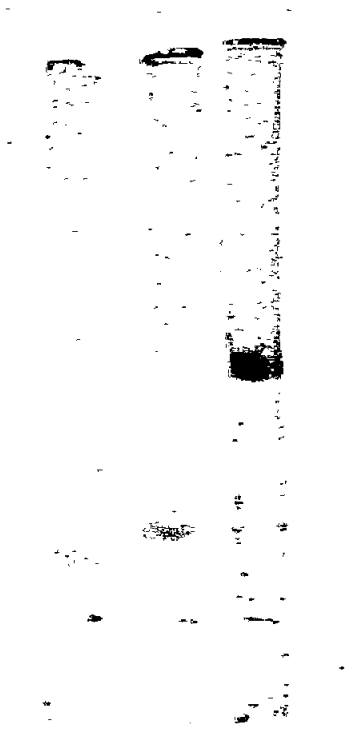

Fig. 2. Gel electrophoresis in the presence of sodium dodecyl sulphate of fractions 82–91 (left), 71–78 (middle) (see fig. 1) and carboxymethylated β-trypsin (right). The proteins migrated from top to bottom.

Essentially the same result was obtained by chromatography of aminoethylated α-trypsin under similar conditions.

3.2. *Amino acid composition of the two chains of α-trypsin*

Table 1 shows the amino acid composition of the two chains of porcine α-trypsin compared with those of bovine α-trypsin [11].

3.3. *Partial amino acid sequence of porcine trypsin*

The amino acid sequence of porcine trypsin starting from the N-terminal was determined up to the thirty-

Table 1
Amino acid composition of the two chains of porcine α-trypsin compared with the corresponding chains of bovine α-trypsin.

Amino acid	NH_2-terminal chain		COOH-terminal chain	
	Pork	Beef[a]	Pork	Beef[a]
Lysine	4.6	5	4.9	9
Histidine	3.6	3	0	0
Arginine	3.7	2	0	0
Aspartic acid	18.1	14	1	8
Threonine	7.9	6	3.8	4
Serine	13.1	19	9.9	15
Glutamic acid	9.2	8	9.1	6
Proline	4.6	3	4.6	5
Glycine	12.4	12	12.2	13
Alanine	12	9	5.0	5
Half-cysteine	4.7[b]	5	6.6[b]	7
Valine	8.9	10	6.9	7
Methionine	1.1[c]	1	1.1[c]	1
Isoleucine	10.1	10	5.9	5
Leucine	11.1	9	7.1	5
Tyrosine	3.6	5	3.8	5
Phenylalanine	3.0	2	1.2	1
Tryptophan	1.7	2	1.6	2

[a] See [11].
[b] Determined as S-aminoethylcysteine.
[c] Determined as methionine sulphone.

first residue with carboxymethylated β-trypsin. Subsequent analysis of the first peak emerging from the Sephadex G-75 column (fig. 1) identified this as the N-terminal portion, of the procine trypsin molecule and confirmed the sequence of this part of the molecule as shown in table 2.

4. Discussion

The separation of the two chains of porcine α-trypsin can be achieved under similar conditions to those used by Schroeder and Shaw [11] for the separation of the two chains of bovine α-trypsin. Gel electrophoresis in the presence of sodium dodecyl sulphate of the separated chains suggests that the molecular weights of the two fragments thus obtained (13,000 and 11,000 daltons) are similar to those obtained in the case of the bovine enzyme and hence that the interchain split in porcine α-trypsin occurs at a similar position in the amino acid sequence. This conclusion is supported by sequence analysis of the separate chains of α-trypsin since as shown in table 2, the sequence of the first portion of the carboxyl terminal chain is very similar to that of the carboxyl terminal chain of the bovine enzyme.

The partial sequence determination of porcine trypsin carried out here suggests that this enzyme is very similar in its primary structure to the bovine enzyme. Hence in the partial sequences described here only six differences in amino acids are to be found between the two enzymes.

Table 2
N-Terminal sequences of the two chains of porcine α-trypsin.

				5				10		Ile				15	
A	Pork	Ile	–Val–	Gly–	Gly–	Tyr–	Thr–	Cys–	Ala–	Ala–	Asn–	Ser–	–	–	Pro–Tyr–Gln
													Val		
	Beef	–	–	–	–	–	–	–	–	–	–	Thr–	Val–	–	–
					20				25					30	
	Pork	Val–	Ser–	Leu–	Asn–	Ser–	Gly–	Ser–	His–	Phe–	Cys–	Gly–	Gly–	Ser–	Leu–Ile
	Beef	–	–	–	–	–	–	–	Tyr	–	–	–	–	–	–
						5				10					
B	Pork	Ser–	Ser–	Gly–	Ser–	Ser–	Tyr–	Pro–	Ser–	Leu–	Leu				
	Beef	–	–	–	–	Thr	–	–	–	Asp–	Val	–			
		132								141					

A is the N-terminal chain and B the C-terminal chain.

The differences in the corresponding sequences of bovine trypsin are shown. In bovine α-trypsin the interchain split is found between residues 131 and 132 [11].

Recently sequences corresponding to residues 1—13 of the N-terminal chain and 1—13 of the C-terminal chain have been quoted from the unpublished work of Ericsson and Hermodson by Hermodson et al. [7]. These sequences were determined from β-trypsin and from α-trypsin without prior separation of the two chains of the latter form. The glutamine residue said to be at position 11 of the carboxyl terminal chain by these authors probably accounts for our present inability to proceed beyond step 10. These authors also report valine and isoleucine in a 2:1 ratio at step 12 of the N-terminal chain. A similar result was obtained in the present work. The sequence 19—29 of the N-terminal chain had been reported earlier by Smith and Liener [12] in studies on the histidine peptides of porcine trypsin.

The determination of the total primary sequence of this enzyme is currently being undertaken by us.

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