REMARKABLE HOMOLOGY ABOUT THE DISULFIDE BRIDGES OF A TRYPSIN-LIKE ENZYME FROM STREPTOMYCES GRISEUS

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SUMMARY

A trypsin-like enzyme which cleaves only the arginine and lysine peptide bonds of the oxidized chains of insulin has been isolated from commercial preparations of pronase. Amino acid analyses revealed the presence of only one histidine and six half-cystine residues. Determination of the amino acid sequences about the three disulfide bridges has demonstrated remarkable homology with three corresponding bridges in bovine pancreatic trypsin. These sequences include both the active serine and histidine as well as the cationic specificity site for trypsin substrates.

It has been shown that Pronase, a commercially available protease preparation from Streptomyces griseus, is a mixture of a number of enzymes (1). One of its components is a BAEE* esterase (2) recently demonstrated to have an active serine sequence Asp-Ser-Gly (3) and a molecular weight of approximately 19,000 (4). Similarity of the enzyme with trypsin has been postulated (5). The specificity of a perhaps similar enzyme from Streptomyces fradiae towards the oxidized chains of insulin and towards lysozyme has been shown to be essentially identical with that of bovine trypsin (6).

MATERIALS AND METHODS

Pronase (B grade) was purchased from the California Corporation for Biochemical Research. The first purification step was essentially as described by Wählby and Engström (3). Fractions containing BAEE esterase activity from

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* Abbreviation: BAEE, benzoyl-L-arginine ethyl ester.

the CM-cellulose column were dialyzed, freeze-dried and applied to a column of Bio-Rex-70 (BioRad Laboratories) (Fig. 1). The yield of purified enzyme was approximately 50 mg. from 5 g. of Pronase. For the specificity studies and amino acid analyses, the enzyme was subjected to an additional passage through the Bio-Rex-70 column. Amino acid analyses were performed in the usual way on a Beckman Model 120B instrument. Half-cystine and methionine were determined as cysteic acid and methionine sulfone (8). Specificity assays were done on the A and B chains of oxidized insulin (Mann Research Laboratories) with an enzyme to substrate molar ratio of 1:200. Incubation was for 1 hour at 30° and pH 8.0 (0.05 M N-ethyl morpholine acetate buffer). The resulting peptides were separated by high voltage electrophoresis on paper and identified by amino acid analysis. Pepsin digests of the enzyme were prepared by an overnight incubation at 37° in 5% formic acid (pepsin 0.5 mg/ml., enzyme 5 mg/ml). The diagonal electrophoretic peptide mapping technique (9) at both pH 6.5 and 1.8 was employed for identification and isolation of the cysteic acid peptides. Their amino acid sequences were determined by the 'Dansyl-Edman' technique (10). In some cases

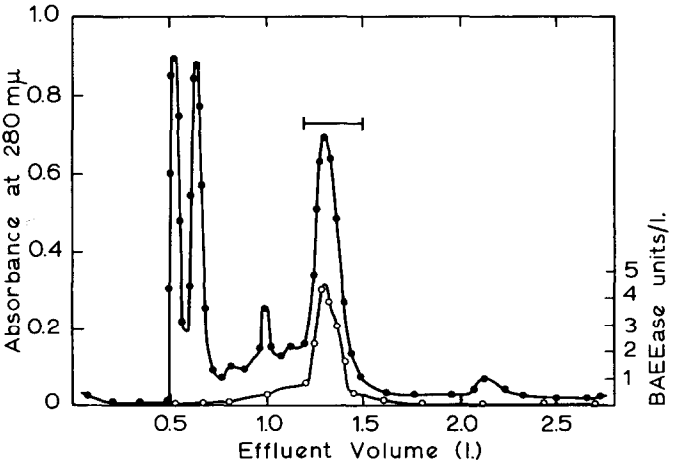

Fig. 1. Elution chromatography of BAEE-ase enriched fraction of Pronase on Bio-Rex-70 resin. Column, 4.5 x 52 cm, was equilibrated with 0.1 M NaOH-cacodylic acid buffer, pH 6.10, and developed at 4°C at a flow rate of 59 ml/hour. BAEE hydrolase activity was determined spectrophotometrically (7). One unit is the amount of enzyme which hydrolyzes one milimole of substrate per second. Fractions were pooled as indicated.

TABLE 1

AMINO ACID COMPOSITION OF THE TRYPSIN-LIKE ENZYME FROM STREPTOMYCES GRISEUS COMPARED WITH TRYPSIN AND α-LYTIC PROTEASE

Amino acid	Trypsin-like enzyme (Leu = 11.0)	Trypsin (15)	α-lytic protease (13)
		Residues	
Lysine	6.5[a]	14	2
Histidine	1.0[a]	3	1
Arginine	8.4[a]	2	12
Aspartic acid	18.1[a]	25	15
Threonine	16.4[b]	10	18
Serine	14.2[b]	34	20
Glutamic acid	17.4[a]	14	13
Proline	8.0[a]	8	4
Glycine	28.4[a]	25	32
Alanine	26.0[a]	14	24
Half-cystine	6.1[d]	12	6
Valine	17.8[c]	17	19
Methionine	2.7[e]	2	2
Isoleucine	8.0[c]	15	8
Leucine	11.0[a]	14	10
Tyrosine	8.2[d]	10	4
Phenylalanine	5.7[a]	3	6
Tryptophan	not determined	4	3

[a] Average of 22-and 70-hour hydrolysates.

[b] Extrapolated to zero time.

[c] Average of 70-hour hydrolysates.

[d] Determined as cysteic acid (8).

[e] Determined as methionine sulfone (8).

fragments were prepared by digestion with the α-lytic protease of Myxobacter (11) The assignment of amides was deduced from the electrophoretic mobilities of the peptides at pH 6.5 (12).

RESULTS AND DISCUSSION

The amino acid composition of the trypsin-like enzyme is compared in Table 1 with those of Myxobacter α-lytic protease (13) and bovine trypsin (14). It is noteworthy that like the α-lytic enzyme, it contains only one histidine and six half-cystine residues.

The specificity study of the enzyme towards the oxidized chains of insulin showed that only the peptide bonds involving the carboxyl groups of arginine and lysine were cleaved. Thus, under the conditions tested, the enzyme had no action on the A chain of insulin. Peptides isolated from the digest of the B chain showed that the enzyme cleaved only on the C-terminal sides of arginine-22 and lysine 29. There was no indication of incomplete proteolysis of these bonds under the conditions tested. A similar specificity has recently

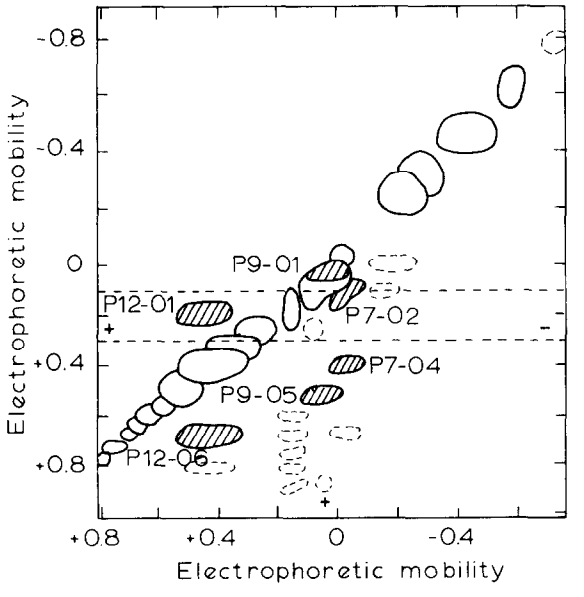

Fig. 2. Diagonal peptide map of cysteic acid peptides from peptic digest of the trypsin-like enzyme. The six cysteic acid peptides are shown as shaded spots. The map was prepared at pH 6.5.

been demonstrated for the trypsin-like enzyme of Streptomyces fradiae (6).

The diagonal peptide map at pH 6.5 of the peptic digest of the enzyme is shown in Fig. 2. The cysteic acid peptide, P9-01, remaining in the neutral region at pH 6.5, was readily identified and isolated from a diagonal map prepared at pH 1.8. All cysteic acid peptides corresponding to the three disulphide bridges and their sequences are shown in Fig. 3. A comparison of these sequences with bovine trypsin (14,15) chymotrypsins A (16) and B (17), porcine elastase (18) and α-lytic protease (13) has shown closest similarity with bovine trypsin. Sequences around both histidine-57 and serine-195 are identical with trypsin over a significant region and are homologous over an even larger area if one includes non-identical but similar residues. Similar but less extensive regions of identity are present in each of the other half-

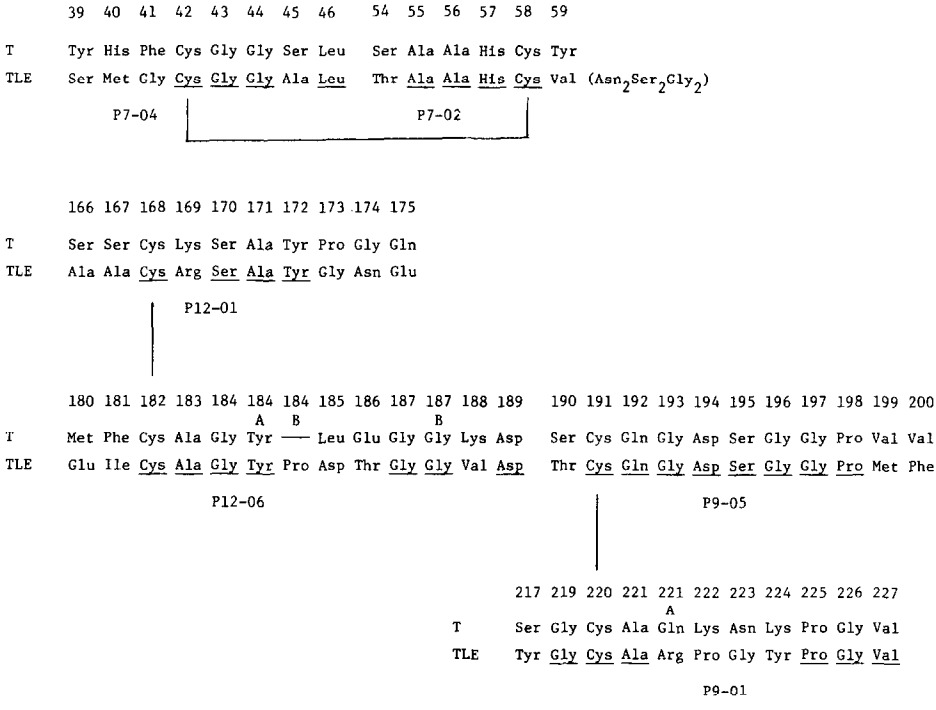

Fig. 3. Amino acid sequences of cysteic acid peptides from peptic digest of the trypsin-like enzyme from Streptomyces griseus (TLE) compared with corresponding sequences of bovine trypsin (T). Identical residues are underlined. The numbering system is based on the sequence of chymotrypsinogen A (16).

cystine sequences. The presence of aspartic acid at position 189 in both the Streptomyces and bovine enzymes is of particular interest with respect to the recent demonstration (19) of the functional role of this residue as the binding site for cationic substrates of trypsin. In other serine proteases (except thrombin) this position is occupied by neutral residues. Thus the trypsin-like enzyme would appear to be similar to bovine trypsin not only in its catalytic mechanism but in its mode of substrate binding.

This comparison of the Streptomyces griseus enzyme and bovine trypsin provides a remarkable example of homology between proteins of widely different phylogenetic origin. Homology based on identity of residues extends over more than 50% of the sequence elucidated to date. It should be emphasized however that these sequences represent only about one quarter of the whole molecule and include structures playing a decisive role in both the substrate binding and catalytic action of the enzyme. This degree of homology will undoubtedly be less in the remaining parts of the sequence to be determined. A further point of interest is the similarity of the trypsin-like enzyme in a number of respects to the α-lytic protease of Myxobacter. Thus its molecular weight is similar, it contains only one histidine and three disulphide bridges linking corresponding regions of the primary structure. The Streptomyces enzyme therefore appears to be intermediate in structural features between those of bovine trypsin on the one hand and α-lytic protease on the other.

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