AMINO ACID SEQUENCE OF CYANOGEN BROMIDE FRAGMENT CB3 OF HOG PEPSIN

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Received November 8th, 1979

On the basis of the knowledge of thermolytic, chymotryptic and substilisin peptides the amino acid sequence was determined of cyanogen bromide fragment CB3 representing the region between methionine residues I and II of pepsin: Thr-Gly-Ile-Leu-Gly-Tyr-Asp-Thr-Val-Gln-Val-Gly-Gly-Ile-Ser-Asp-Thr-Asn-Gln-Ile-Phe-Gly-Leu-Ser-Glu-Thr-Glu-Pro-Gly-Ser-Phe-Leu-Tyr-Tyr-Ala-Pro-Phe-Asp-Gly-Ile-Leu-Gly-Leu-Ala-Tyr-Pro-Ser-Ile-Ser-Ala-Ser-Gly-Ala-Thr-Pro-Val-Phe-Asp-Asn-Leu-Trp-Asp-Gln-Gly-Leu-Val-Ser-Gln-Asp-Leu-Phe-Ser-Val-Tyr-Leu-Ser-Ser-Asn-Asp-Asp-Ser-Gly-Ser-Val-Val-Leu-Leu-Gly-Gly-Ile-Asp-Ser-Ser-Tyr-Tyr-Thr-Gly-Ser-Leu-Asn-Trp-Val-Pro-Val-Ser-Val-Glu-Gly-Tyr-Trp-Gln-Ile-Thr-Leu-Asp-Ser-Ile-Thr-Met.

In sequential studies on hog pepsin, carried out in this laboratory, fragments CB1 to CB6 were prepared by cyanogen bromide cleavage of S-sulfo-pepsin[1]. Tryptic digestion of aminoethylated pepsin[2] was employed as an alternative fragmentation procedure. The sequential analysis of the cyanogen bromide fragments[3-5] provided the basic information for the determination of the complete and by now published[6] amino acid sequence of the enzyme. As a result of incomplete cleavage at the carboxyl side of the first methionine residue (bond ..Met-Thr..) a longer fragment CB2, corresponding to the region of pepsin extending from the N-terminus to the methionine residue II and comprising segments corresponding to fragments CB4 and CB3 (ref.[1]), was isolated in addition to the products expected. For these reasons we were able to utilize for the investigation of the region between methionine residue I and II of pepsin both fragment CB3 and CB2, as well as tryptic fragment RAEP-tA 22 of aminoethylated pepsin[2], also comprising this region.

This paper provides experimental data on the determination of the amino acid sequence of the remaining cyanogen bromide fragment CB3 representing the region of the polypeptide chain between methionine residues I and II.

EXPERIMENTAL

Material

Cyanogen bromide fragments CB2 and CB3 were prepared in earlier work[1,5]. The chemicals and materials used are described in earlier papers cited below.

TABLE I

Amino Acid Composition of Thermolytic Peptides Derived from the Region of Fragment CB3.

The peptides were analyzed after 20-h hydrolysis; the values are not corrected. None of the peptides contained lysine, histidine, arginine, or half-cystine.

Designation of peptide	Number of amino acid residues													
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	Hse[a]	Trp
Th1	—	2·0	1·2	—	—	3·0	—	—	—	—	—	—	0·8	—
Th2	—	0·9	—	—	—	1·0	—	—	—	—	—	—	0·7[b]	—
Th3	1·0	0·9	—	—	—	1·0	—	—	1·0	1·0	0·8	—	—	—
Th4	—	—	—	—	—	1·0	—	—	0·8	1·1	—	—	—	—
Th5	1·0	1·0	—	—	—	—	—	—	—	—	0·8	—	—	—
Th6	—	—	—	1·0	—	—	—	1·0	—	—	—	—	—	—
Th7	—	—	—	—	—	1·9	—	1·0	—	—	—	—	—	—
Th8	2·0	0·9	1·0	1·0	—	—	—	—	1·0	—	—	—	—	—
Th9	—	—	—	—	—	1·0	—	—	0·9	—	—	1·1	—	—
Th10	—	1·0	1·9	2·0	1·0	1·1	—	—	—	1·0	—	1·0	—	—
Th11	—	1·1	2·0	1·9	1·1	1·2	—	—	—	0·7	—	—	—	—
Th12	—	—	—	—	—	—	—	—	—	1·0	1·0	—	—	—
Th13	—	—	—	—	1·0	—	1·0	—	—	—	0·7	—	—	—
Th14	—	—	—	—	1·0	—	1·0	—	—	—	—	—	—	—
Th15	1·0	—	—	—	—	1·1	—	—	—	—	—	1·0	—	—
Th16	—	—	—	—	—	1·0	—	—	0·8	1·1	—	—	—	—
Th17	1·1	—	—	—	—	1·9	—	—	0·8	1·0	—	1·1	—	—
Th18	—	—	0·9	—	1·0	—	1·0	—	—	1·0	0·9	—	—	—
Th19	—	—	1·0	—	1·0	—	1·0	—	—	—	0·9	—	—	—
Th20	—	1·3	1·8	—	1·0	1·1	2·0	—	0·9	—	—	—	—	—
Th21	2·1	1·0	1·8	—	0·8	1·0	1·8	0·9	0·9	—	—	0·9	—	—
Th22	—	—	1·8	—	—	1·3	1·0	—	0·9	—	—	—	—	—
Th23	2·1	1·0	—	—	1·2	—	0·8	1·0	—	—	—	0·9	—	—
Th24	2·2	—	—	—	—	—	—	1·0	—	—	—	1·0	—	—
Th25	1·1	—	—	1·0	—	1·1	—	—	—	1·0	—	—	—	+[c]
Th26	1·2	—	1·0	1·0	—	—	—	1·1	—	1·8	—	—	—	—
Th27	1·1	—	1·0	1·1	—	—	—	1·0	—	0·9	—	—	—	—
Th28	1·0	—	0·9	1·0	—	—	—	0·8	—	—	—	—	—	—
Th29	—	—	1·0	—	—	—	—	—	—	—	—	1·0	—	—
Th30	—	—	—	—	—	—	—	1·0	—	—	0·9	—	—	—
Th31	3·3	—	3·8	—	—	1·2	—	1·7	—	2·0	—	—	—	—
Th32	—	—	—	—	—	2·0	—	—	—	1·0	—	—	—	—
Th33	1·3	—	1·9	—	—	2·1	—	—	0·9	0·8	1·1	—	—	—
Th34	1·1	—	1·9	—	—	—	—	—	1·0	—	0·8	—	—	—
Th35	—	1·0	1·0	—	—	1·0	—	—	—	—	0·8	—	—	—
Th36	1·0	—	—	—	1·1	—	—	1·0	—	0·9	—	—	—	+[c]
Th37	—	—	0·8	—	—	—	—	1·0	—	—	—	—	—	—
Th38	—	—	0·9	1·1	—	1·0	—	2·0	—	—	—	—	—	—

TABLE I
(*Continued*)

Designation of peptide	Number of amino acid residues													
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	Hse[a]	Trp
Th39	—	—	—	1·9	—	1·1	—	1·0	—	—	0·7	—	—	+[c]
Th40	—	—	—	1·2	—	1·1	—	1·0	—	—	0·6	—	—	—
Th41	—	1·2	—	—	—	—	—	—	1·0	—	—	—	—	—
Th42	1·1	—	1·0	—	—	—	—	—	—	0·9	—	—	—	—
Th43	1·2	1·9	—	1·1	—	1·1	—	—	1·0	—	—	—	0·8[b]	—

[a] Hse denotes homoserine; [b] methionine was determined in the peptide; [c] the tryptophan content was determined qualitatively only.

Methods

For chymotryptic digestion lyophilized fragment CB3 (350 mg) was dissolved in 30 ml of water made alkaline with 0·1M-NH_4HCO_3 (red color of phenol red added to the solution as an indicator). The substrate was cleaved by two portions of chymotrypsin (final weight ratio of enzyme to substrate 1 : 50) 4 h at 37°C. The digest was fractionated by gel filtration on a column (65 × × 5 cm) of Sephadex G-15 equilibrated with 0·05M-NH_4HCO_3. Fractions (20 ml/15 min) were evaluated by paper chromatography of aliquots (0·2 ml) in the system 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12, v/v) (ref.[7]). Paper chromatography in this system and two electrophoretic procedures on Whatman No 3 or 3 MM paper were also used for the final purification of the peptides. The separation at pH 1·9 in the system formic acid–acetic acid–water (50 : 150 : 800, v/v) at 4000 V was carried out in the horizontal arrangement in the apparatus constructed by Prusík and Štěpánek[8]. The separation at pH 5·6 in the system pyridine–acetic acid–water (5 : 1 : 494, v/v) was performed at 1 500 V in the vertical arrangement in the apparatus designed by Mikeš[9].

The preparation and separation of the chymotryptic digest of fragment CB2 has been described in the preceding communication[10], as well as the treatment of the chymotryptic digest of S-sulfo-pepsin[11]. The investigation of the thermolysin digest of fragment CB2 (ref.[10]) and of the thermolysin digest of S-sulfo-pepsin[12] have also been described before. The amino acid compositions and sequences of those peptides from the digests which fall into the region of fragment CB3 are given in Tables I—IV. Peptides obtained in duplicate are listed once only, in order of their location in the sequence of fragment CB3. Subtilisin peptides, given in the scheme in Fig. 1, were isolated from the digest of tryptic fragment RAEP-tA 22 of aminoethylated pepsin[13].

Amino acid analyses were performed on 20- and 70-h hydrolysates (at 110°C) by the method of Spackman and coworkers[14] as modified by Benson and Patterson[15] in Model 6020 Amino Acid Analyzer, manufactured by the Instrument Development Workshop, Czechoslovak Academy of Sciences, Prague. Homoserine lactone was converted[16] to homoserine before the analysis. The amino acid sequences of peptides were determined by Edman degradation[17]; the fenylthiohydantoins were analyzed by thin-layer chromatography on silica gel. In certain cases the chymo-

TABLE II

Sequence of Thermolytic Peptides Derived from the Region of Fragment CB3

Designation of peptide	Amino acid sequence of peptide
Th1	Gly-Thr-Gly-Ser-Hse-Thr-Gly
Th2	Met-Thr-Gly
Th3	Ile-Leu-Gly-Tyr-Asp-Thr
Th4	Ile-Leu-Gly
Th5	Tyr-Asp-Thr
Th6	Val-Gln
Th7	Val-Gly-Gly
Th8	Ile-Ser-Asp-Thr-Asn-Gln
Th9	Ile-Phe-Gly
Th10	Leu(Ser,Glx,Thr,Glx,Pro,Gly,Ser,Phe)
Th11	Leu(Ser,Glx,Thr,Glx,Pro,Gly,Ser)
Th12	Leu-Tyr
Th13	Tyr-Ala-Pro
Th14	Ala-Pro
Th15	Phe(Asp, Gly)
Th16	Ile-Leu-Gly
Th17	(Phe, Asx, Gly, Ile, Leu, Gly)
Th18	Leu-Ala-Tyr-Pro-Ser
Th19	Ala-Tyr-Pro-Ser
Th20	Ile-Ser-Ala-Ser-Gly-Ala-Thr-Pro
Th21	Ile(Ser,Ala,Ser,Gly)(Ala,Thr,Pro,Val,Phe,Asx,Asx)
Th22	(Ile,Ser,Ala,Ser,Gly)
Th23	(Ala,Thr,Pro,Val,Phe,Asx,Asx)
Th24	(Val,Phe,Asx,Asx)
Th25	Leu-Trp-Asp-Gln-Gly
Th26	Leu-Val-Ser-Gln-Asp-Leu
Th27	Leu(Val,Ser,Glx,Asx)
Th28	Val(Ser,Glx,Asx)
Th29	Phe-Ser
Th30	Val-Tyr
Th31	Leu-Ser-Ser-Asn-Asp-Asp(Ser,Gly,Ser,Val,Val,Leu)
Th32	Leu-Gly-Gly
Th33	Leu(Gly,Gly,Ile,Asx,Ser,Ser,Tyr)
Th34	Ile(Asx,Ser,Ser,Tyr)
Th35	Tyr-Thr-Gly-Ser
Th36	Leu-Asn-Trp-Val-Pro
Th37	Val-Ser
Th38	Val(Ser,Val,Glu,Gly)
Th39	Val(Glx,Gly,Tyr,Trp)Gln
Th40	Val-Glu(Gly,Tyr)
Th41	Ile-Thr
Th42	Leu-Asp-Ser
Th43	Ile(Thr,Met,Asx,Gly,Glx,Thr)

tryptic peptides were assigned C-terminal end groups with respect to the specificity of cleavage by this enzyme. Other details of the isolation and characterization of the peptides are described in the preceding papers[11-13].

RESULTS AND DISCUSSION

Fragment CB3 is formed by cyanogen bromide cleavage of S-sulfo-pepsin, at methionine residues I and II (ref.[1]). As a result of incomplete cleavage of the bond involving methionine I this part of the pepsin chain is contained also in the product cleaved

TABLE III

Amino Acid Composition of Chymotryptic Peptides Derived from the Region of Fragment CB3

The peptides were analyzed after 20 h hydrolysis; the values are not corrected. None of the peptides contained lysine, histidine, arginine, or half-cystine.

Designation of peptide	Number of amino acid residues													
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	Hse	Trp
C1	—	1·9	1·0	—	—	3·6	—	—	1·0	1·0	0·5	—	1·2	—
C2	—	2·0	1·0	—	—	2·9	—	—	1·0	1·0	—	—	1·1	—
C3	3·1	2·0	1·0	2·2	—	2·2	—	2·0	1·7	—	—	0·8	—	—
C4	—	1·1	2·0	2·0	1·1	2·0	—	—	—	1·1	—	1·0	—	—
C5	—	1·0	2·1	2·0	1·0	2·0	—	—	—	1·9	0·8	1·0	—	—
C6	—	1·0	1·7	2·0	1·0	2·0	—	—	—	2·1	—	1·0	—	—
C7	—	—	—	—	—	—	—	—	—	1·0	0·8	—	—	—
C8	—	—	—	—	1·1	—	1·0	—	—	—	1·0	1·0	—	—
C9	1·1	—	—	—	1·1	1·1	1·1	—	0·9	1·0	0·6	1·0	—	—
C10	—	—	—	—	1·0	—	1·0	—	—	—	—	0·9	—	—
C11	1·0	—	—	—	—	2·1	—	—	0·9	2·0	—	—	—	—
C12	4·8	1·4	4·0	2·5	2·1	2·6	3·0	2·2	1·2	3·4	0·9	2·1	—	+[a]
C13	4·1	1·2	1·9	2·3	1·2	2·2	1·8	2·0	—	3·0	—	1·9	—	+[a]
C14	2·1	—	1·3	1·7	—	1·1	—	1·1	—	1·9	—	0·8	—	—
C15	1·1	—	1·1	1·0	—	—	—	0·9	—	0·9	—	0·8	—	—
C16	—	—	0·9	—	—	—	—	1·0	—	—	0·9	—	—	—
C17	3·0	—	3·7	—	—	1·1	—	1·5	—	2·0	—	—	—	—
C18	1·3	—	2·0	—	—	2·0	—	—	1·0	1·1	1·6	—	—	—
C19	1·1	0·9	1·0	—	—	1·0	—	—	—	1·0	—	—	—	+[a]
C20	—	—	0·9	0·9	1·0	1·0	—	2·8	—	—	1·0	—	—	+[a]
C21	1·0	1·0	1·0	—	—	—	—	—	0·9	—	—	—	1·2	—

[a] The tryptophan content was determined qualitatively only.

TABLE IV

Sequence of Chymotryptic Peptides Derived from the Region of Fragment CB3

Designation of peptide	Amino acid sequence of peptide
C1	Gly-Thr-Gly(Ser,Hse,Thr,Gly)(Ile,Leu,Gly)Tyr
C2	Gly(Thr,Gly,Ser,Hse,Thr,Gly,Ile)Leu
C3	Asp-Thr-Val-Gln-Val-Gly-Gly-Ile-Ser-Asp(Thr,Asx,Glx,Ile)Phe
C4	Gly-Leu-Ser-Glu-Thr-Glu-Pro-Gly-Ser-Phe
C5	Gly(Leu,Ser,Glx,Thr,Glx,Pro,Gly,Ser)Phe-Leu-Tyr
C6	(Gly,Leu,Ser,Glx,Thr,Glx,Pro,Gly,Ser,Phe,Leu)
C7	Leu-Tyr
C8	Tyr-Ala-Pro-Phe
C9	(Tyr,Ala,Pro,Phe,Asx,Gly,Ile,Leu)
C10	(Ala,Pro)Phe
C11	Asp-Gly-Ile-Leu-Gly-Leu
C12	Ala-Tyr-Pro-Ser-Ile-Ser-Ala(Ser_2,Gly_2,Ala,Thr,Pro,Val_2,Phe_2,Asx_4,Leu_3,Trp, ,Glx_2)
C13	Ala-Ser-Gly-Ala-Thr-Pro-Val-Phe-Asp(Asx,Leu,Trp,Asx,Glx,Gly,Leu,Val,Ser, ,Glx,Asx,Leu,Phe)
C14	Asp-Gln-Gly-Leu-Val-Ser-Gln-Asp-Leu-Phe
C15	(Val,Ser,Glx,Asx Leu)Phe
C16	Ser-Val-Tyr
C17	Leu-Ser-Ser-Asn-Asp-Asp-Ser-Gly-Ser-Val-Val-Leu
C18	(Leu,Gly,Gly,Ile,Asx,Ser,Ser,Tyr)Tyr
C19	Thr-Gly-Ser-Leu-Asn-Trp
C20	(Val,Pro,Val,Ser,Val,Glu,Gly,Tyr)Trp
C21	Asp-Ser-Ile-Thr-Hse

TABLE V

Amino Acid Composition of Fragment CB3

The values are given as number of amino acid residues in the molecule of the fragment. Arginine, lysine, histidine, a half-cystine were not found in the fragment.

Fragment	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	Hse	Trp
CB3 (ref.[5])	14·0	7·8[a]	16·9[a]	8·2	4·6	14·6	4·1	9·8[b]	7·9[b]	12·7	7·4	5·0	0·8	+[c]
In sequence of CB3	14	8	17	8	5	15	4	10	8	13	8	5	1	3

[a] Values extrapolated to zero time of hydrolysis; [b] values determined after 70-h hydrolysis; [c] the tryptophan content was determined qualitatively only.

incompletely (fragment CB2) in which the N-terminal fragment CB4 is linked to fragment CB3 following in the chain. Fragment CB3 was obtained during the basic fractionation of the cyanogen bromide digest by gel filtration; this preparation containing contaminants was characterized[1] by the N-terminal sequence Thr-Gly-Ile--Leu-Gly-Tyr-... and by the C-terminal sequence Ser(Ile,Thr)Hse. The carboxyl terminus of fragment CB3 is identical with the C-terminus of fragment CB2, determined as Ile-Thr-Hse. The isolation of the corresponding homoserine peptide from the chymotryptic digest of fragment CB2 (ref.[10]) permitted the carboxyl terminal region of fragment CB3 and CB2 to be formulated as Asp-Ser-Ile-Thr-Hse (ref.[1]). Fragment CB3 was purified by ion exchange chromatography[5]; the amino acid composition of this preparation is given in Table V. The tryptophan content (Table V) was not determined directly, it is known, however, from the analysis of fragment RAEP-tA 22 (2·9 residues, ref.[2]), whose terminal parts exceeding fragment CB3 do not contain tryptophan[5]. The N-terminal sequence of the fragment, Thr-Gly-Ile--Leu-Gly-Tyr-..., was verified by manual Edman degradation of pure fragment CB3. Because of the absence of lysine, arginine, and half-cystine (convertible into the aminoethylcysteine residue, sensitive to trypsin) in the part of the pepsin chain overlapping fragment CB3 this part of the chain was not cleaved by tryptic digestion of aminoethylated pepsin and a large fragment RAEP-tA 22 (ref.[2]) derived from this site was isolated. For the same reason tryptic hydrolysis could not be employed for sequential investigation of fragment CB3.

The amino acid sequence of the fragment (Fig. 1) was derived mainly from information afforded by chymotryptic, thermolytic, and subtilisin peptides. Data on the chymotryptic peptides obtained by the digestion of fragment CB3 were complemented by the alignment of peptides from the chymotryptic digest[10] of fragment CB2 (after elimination of peptides incorporated into the sequence[5] of fragment CB4) and, knowing the structures of the remaining cyanogen bromide fragments[3-5], also by selected peptides from the chymotryptic hydrolysate of S-sulfo-pepsin[11]. Likewise, after the determination of the sequence of fragment CB4, the peptides remaining from the thermolytic digest[10] of fragment CB2 could be unambiguously incorporated into the region of CB3 and it became possible to also align selected peptides from the thermolysin digest of S-sulfo-pepsin[12]. Tryptic fragment RAEP-tA 22 (ref.[2]) served as a source for the preparation of subtilisin peptides. The subtilisin digest is described in more detail in the adjoining paper[13]; only selected peptides from this digest, complementing data obtained with other digests, are shown in the scheme in Fig. 1. In a number of cases the sequential characterization of the peptides (Table II and IV) was omitted wherever the information had been obtained with other peptides.

Unambiguous sequential overlaps (Fig. 1) permitted the amino acid sequence of the fragment to be arranged into four sections:

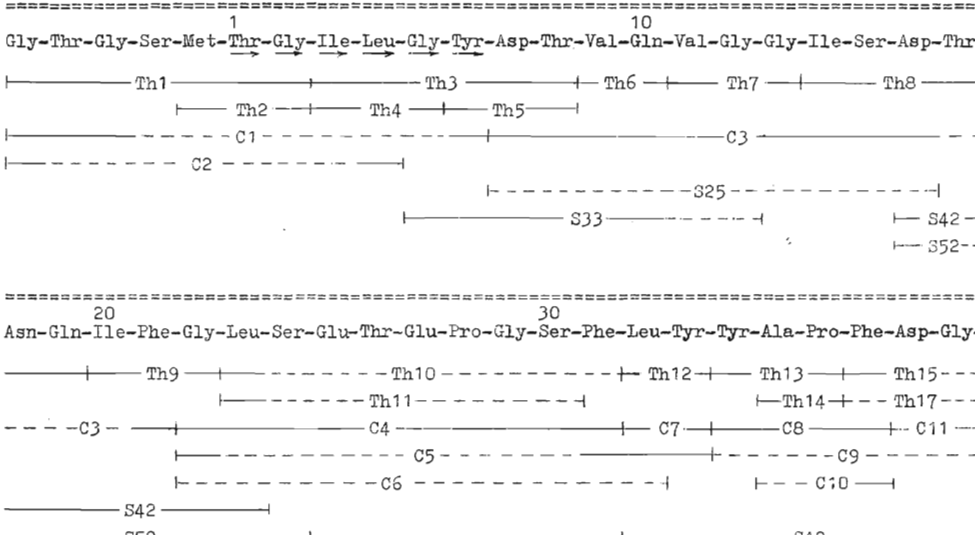
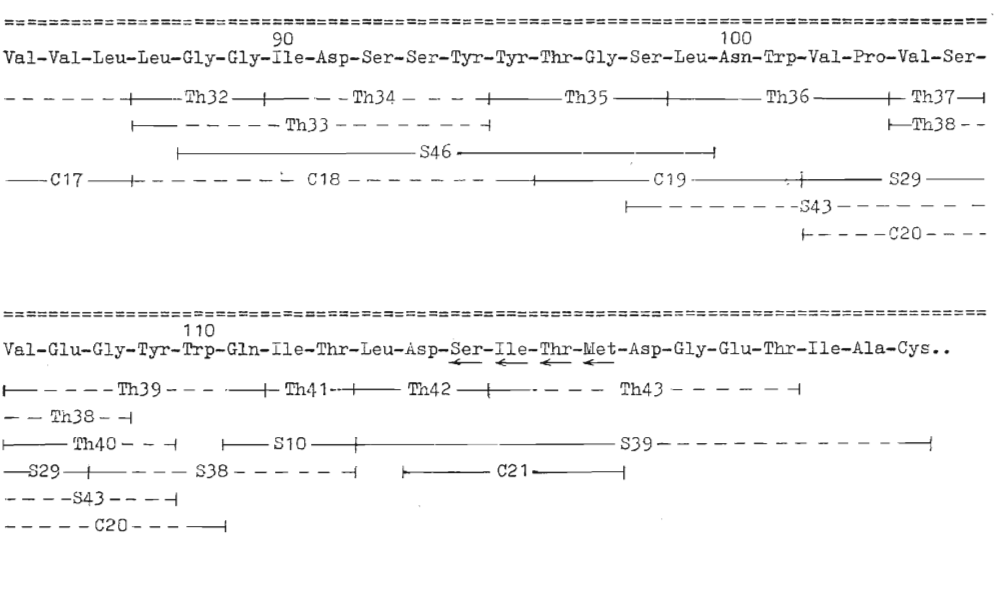


FIG. 1

Amino Acid Sequence of Fragment CB3

The peptides are denoted by lines, completely determined sequences by full lines, incomplete sequences by broken lines. Symbols "Th" and "C" stand for thermolytic and chymotryptic peptides, respectively. The subtilisin peptides are marked "S" and numbered according to the adjoining paper[13]. The Edman degradation steps are marked →, the results of carboxypeptidase A cleavage ←.

40
Ile-Leu-Gly-Leu-Ala-Tyr-Pro-Ser-Ile-Ser-Ala-Ser-Gly-Ala-Thr-Pro-Val-Phe-Asp-Asn-Leu-Trp-
50
60
Th16
Th18
Th20
Th24
Th25
Th17
Th19
Th21
C11
Th22
Th23
C9
C12
S11
S36
S15
S50
S49
C13
70
80
Asp-Gln-Gly-Leu-Val-Ser-Gln-Asp-Leu-Phe-Ser-Val-Tyr-Leu-Ser-Ser-Asn-Asp-Asp-Ser-Gly-Ser-
Th25
Th26
Th29
Th30
Th31
Th27
Th28
C12
C16
C17
C14
S50
C15
S54
C13


90
100
Val-Val-Leu-Leu-Gly-Gly-Ile-Asp-Ser-Ser-Tyr-Tyr-Thr-Gly-Ser-Leu-Asn-Trp-Val-Pro-Val-Ser-
Th32
Th34
Th35
Th36
Th37
Th33
Th38
S46
C17
C18
C19
S29
S43
C20
110
Val-Glu-Gly-Tyr-Trp-Gln-Ile-Thr-Leu-Asp-Ser-Ile-Thr-Met-Asp-Gly-Glu-Thr-Ile-Ala-Cys..
Th39
Th41
Th42
Th43
Th38
Th40
S10
S39
S29
S38
C21
S43
C20

I	Thr-Gly-Ile ..... Ser-Val-Tyr	(res.	1–74)
II	Leu-Ser-Ser ..... Val-Val-Leu	(res.	75–86)
III	Leu-Gly-Gly ..... Gln-Ile-Thr	(res.	87–113)
IV	Leu-Asp-Ser-Ile-Thr-Hse	(res.	114–119)

We were not able to join together the above regions by overlapping peptides. The total sum of amino acid residues in these noneverlapping sequential regions equals the amino acid composition of fragment CB3 (Table V). The peptides from the individual enzymatic digests comply with this arrangement of sequential regions and do not suggest the existence of overlaps or, by contrast, a loss of peptides where these regions are linked to one another (residues 74/75, 86/87, and 113/114). The N-terminal sequence of region I is identical to the N-terminal sequence of fragment CB3; sequence IV, containing the homoserine residue, represents the carboxyl terminus of the fragment. The order of the middle regions II and III was established from the results of carboxypeptidase A degradation of fragments CB3 and CB2 (ref.[1]). During prolonged cleavage the quantity of threonine and isoleucine liberated exceeds the quantity of C-terminal homoserine; it may thus be assumed that the short sequential region IV (Leu-Asp-Ser-Ile-Thr-Hse) is preceded by a threonine and isoleucine residue. This assumption is in accordance with the carboxyl terminus of region III, ..Gln-Ile-Thr, since region II is C-terminated by ..Val-Val-Leu. As evidence supporting the location of region II after the N-terminal sequence I may be adducted the fact that the bond at the amino terminus of leucine No 75 is susceptible to cleavage by both chymotrypsin and bromosuccinimide[18]. This observation requires the presence of a tyrosine residue at the amino side of leucine 155 since the complete knowledge of the sequence around the three tryptophans of fragment CB3 excludes this residue as an alternative. Likewise, the sensitivity of the bond between residues 86 and 87 to chymotrypsin is compatible with the proposed bond Leu(86)-Leu(87).

The sequence of fragment CB2A, determined by Sepulveda and coworkers[19] is in complete agreement with our sequence of fragment CB3 (Fig. 1) which has already been reported in the paper describing the complete amino acid sequence of hog pepsin[6]; partial data pertaining to this part of the molecule were also published by Ostoslavskaya and coworkers[20]. Likewise the results reported by Revina and coworkers[21], accounting for residues 100–119 of this fragment, confirm our sequence.

We thank Mrs J. Viková and Mrs E. Bulantová for careful technical assistance, Mr K. Grüner and Mrs A. Čermáková for the Edman degradation. We are indebted to Mr J. Zbrožek, Miss V. Himrová, and Mrs E. Držková for the amino acid analyses of peptides.

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Translated by the author (V. K.).