AMINO ACID SEQUENCE OF THE PEPTIDE RELEASED FROM BOVINE FACTOR XIII FOLLOWING ACTIVATION BY THROMBIN

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SUMMARY: The amino acid sequence of the peptide released during the conversion of bovine Factor XIII to the active enzyme by thrombin was determined. It contains N-terminal N-acetylserine and a total of 37 residues. The bovine peptide differs from the corresponding human peptide. There are 5 amino acid replacements and one deletion in the human peptide.

Factor XIII is the proenzyme of a transglutaminase found in mammalian plasma and platelets (1). It is composed of two pairs of subunit polypeptides, a and b, with molecular weights of about 75,000 and 85,000, respectively (2-4). Upon activation of Factor XIII by thrombin, a small peptide with a molecular weight of about 4,000 is released from the N-terminal

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portion of the molecule (5, 6). The bovine peptide has a total of 37 amino acid residues, a masked N-terminal end and C-terminal arginine (6). This paper contains the complete amino acid sequence of the peptide and a comparison with the corresponding human peptide recently reported by Takagi and Doolittle (7).

MATERIALS AND METHODS

Factor XIII was purified from citrated fresh bovine plasma (4). Bovine thrombin was purified (8) from a commercial preparation (Mochida Pharmaceutical Co. Ltd., Tokyo). Thermolysin was a generous gift from Dr. K. Morihara, Shionogi Research Laboratory, Co., Osaka. Carboxypeptidase A (DFP-treated) was obtained from Worthington Biochemical Corp.. An acylamino acid releasing enzyme purified from rat liver was kindly provided by Dr. S. Tsunazawa of this institute (9). 1-Dimethylamino-naphthalene-5-sulfonyl (DNS) chloride and standard DNS-amino acids were obtained from Seikagaku Kogyo Co., Ltd., Tokyo.

The peptide was released by activating 90 mg purified Factor XIII with 300 NIH units thrombin (6). The peptide was isolated using Sephadex G-75 and 10 % acetic acid. Digestions of the purified peptide with TPCK-trypsin (twice crystallized) (10) and α-chymotrypsin (three times crystallized) were made at 37°C in 0.2 M ammonium bicarbonate buffer, pH 8.0, for 4 hours. The peptide was also digested at 40°C in the same buffer with thermolysin for 24 hours, using the weight ratio of enzyme to substrate of 1 : 50. The release of C-terminal residues by carboxypeptidase A was effected in the same buffer at 40°C.

Removal of N-acetylserine from the N-terminal chymotryptic fragment of tryptic peptide II was achieved by incubating it with rat liver acylamino acid releasing enzyme at 40°C in 0.2 M ammonium bicarbonate buffer, pH 8.0, for 24 hours, using the weight ratio of enzyme to substrate of 2 : 1. The digests were either separated by gel-filtration on a Sephadex G-25 (superfine) column (1.2 x 95 cm), using 10 % acetic acid as the eluant or by chromatography on a Dowex 50 x 2 column (0.4 x 4.0 cm), using a stepwise elution with pyridine-acetate buffer (pH 3.05 to 5.5). The separation of the fragments was also performed by high voltage paper electrophoresis with pyridine acetate buffer, pH 3.4. The elution of the peptide from paper was made with 10 % acetic acid containing 5 % pyridine. Amino acid sequences of the peptide were determined by a slight modification (12) of the Edman-dansyl method (11). DNS-amino acids were identified by two-dimentional thin layer chromatography on polyamide sheets, using the solvents reported (12, 13). The N-acetyl-group of the N-terminal chymotryptic fragment of tryptic peptide II was characterized as its 1-acetyl-2-dansylhydrazine derivative (14). N-acetylserine was identified by comparison with a synthetic sample using thin layer electrophoresis on a cellulose plate. It was also identified as free serine after hydrazinolysis. The amino acid compositions of peptides were determined with an amino acid analyzer,

Table 1. Amino acid composition of tryptic fragments

Amino acid	Whole peptide	T-I	T-II	T-III
	——— Residues per mole ———			
Aspartic acid	4.0(4)	3.8(4)		
Threonine	2.8(3)	1.8(2)	1.0(1)	
Serine	3.4(4)	1.1(1)	2.8(3)	
Glutamic acid	3.8(4)	2.9(3)	0.9(1)	
Proline	4.7(5)	5.0(5)		
Glycine	3.9(4)	1.2(1)	3.0(3)	
Alanine	3.9(4)	3.1(3)	1.3(1)	
Valine	2.3(2)	2.1(2)		
Isoleucine	1.1(1)	1.0(1)		
Leucine	2.0(2)	1.9(2)		
Phenylalanine	1.0(1)		0.9(1)	
Arginine	2.8(3)	1.1(1)	1.0(1)	1.0
Total	37	25	11	1
Yield (%)		87	90	41

Model JLC-5AH, Japan Electron Optics Lab., Ltd., according to the method of Spackman (15). The presence of amides of glutamic and aspartic acid residues was inferred from the electrophoretic behavior of the peptide containing these amino acids.

RESULTS

Fragments isolated from tryptic and chymotryptic digests

Tryptic digestion of the whole peptide (0.28 μmole) yielded three major fragments, T-I, T-II and T-III (free arginine), which were separable by gel-filtration on a Sephadex G-25 column. The largest fragment, T-I, consisted of 25 amino acid residues (Table 1) and the N-terminal sequence of first 13 residues was established as shown in Table 2. The fragment, T-II, contained 11 residues and an N-terminus unreactive to dansyl-chloride. The chymotryptic digestion of this fragment yielded a ninhydrin-negative octapeptide with C-terminal phenylalanine (T-II-C-1), and a tripeptide of Gly-

Table 2. Partial or complete amino acid sequences of tryptic, chymotryptic and thermolytic fragments

Fragments	Partial or complete amino acid sequences
T-I	Ala→-Ile→-Pro→-Pro→-Asx→-Thr→-Ser→-Asx→-Ala→-Ala→-Glx→-Asx→-Asx→-(Pro, Pro, Thr, Val, Glx, Leu, Glx, Gly, Leu, Val, Pro, Arg)
T-II-C-1	Ac-(Ser, Glx, Ser, Ser, Gly, Thr)Ala←-Phe←
T-II-C-1A	Ac-Ser
T-II-C-1B	Glu→-Ser→-Ser→-Gly→-Thr→-Ala→-Phe→
T-II-C-2	Gly→-Gly→-Arg→
T-III	Arg→
C-VI	Gly→-Gly→-Arg→-Arg→
T-I-C-1	Ala→-(Ile, Pro, Pro, Asx, Thr, Ser, Asx, Ala, Ala, Glx, Asx, Asx, Pro, Pro, Thr)Val←-Glu←-Leu←
T-I-C-2	Gln→-Gly→-Leu→-(Val, Pro, Arg)
T-I-TL-3	Ala→-Ile→-Pro→-Pro→-Asn→-Thr→-Ser→-Asn→
T-I-TL-5	Ala→-Ala→-Glu→-Asp→-Asp→-Pro→-Pro→-Thr→
T-I-TL-4	Val→-Glu→
T-I-TL-2	Leu→-Gln→-Gly→
T-I-TL-1	Leu→-Val→-Pro→-Arg→

The amino acids identified by Edman-dansyl and carboxypeptidase A methods were indicated by arrows. For the designation of the various peptides the following abbreviations were used: tryptic, T; chymotryptic, C and thermolytic, TL.

Gly-Arg (T-II-C-2), which were separable on a Dowex 50 x 2 column. The ninhydrin-negative octapeptide was subsequently treated with the rat liver acylamino acid releasing enzyme and two fragments were isolated by gel-filtration on a Sephadex G-15 column (1.0 x 45 cm). One fragment (T-II-C-1A) was identified as free N-acetylserine and the sequence of the other fragment (T-II-C-1B) was established as shown in Table 2. These results

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                1                   5                  10
Bovine  N-Ac-Ser-Glu-Ser-Ser-Gly-Thr-Ala-Phe-Gly-Gly-Arg-Arg-

Human   N-Ac-Ser-Glu-Thr-Ser-Arg-Thr-Ala-Phe-Gly-Gly-Arg-Arg-

          15                  20                  25
Ala-Ile-Pro-Pro-Asn-Thr-Ser-Asn-Ala-Ala-Glu-Asp-Asp-Pro-Pro-Thr-

Ala-Val-Pro-Pro-Asn-Asn-Ser-Asn-Ala-Ala-Glu-Asp-Asp-Leu-Pro-Thr-

     30                  35
Val-Glu-Leu-Gln-Gly-Leu-Val-Pro-Arg-OH

Val-Glu-Leu-Gln-Gly-   -Val-Pro-Arg-OH
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Fig. 1. Amino acid sequence of the peptide released following activation of bovine Factor XIII by thrombin and its comparison with the human peptide determined by Takagi and Doolittle (7).

indicated the following structure for fragment T-II: N-acetyl-Ser-Glu-Ser-Ser-Gly-Thr-Ala-Phe-Gly-Gly-Arg.

To obtain a second set of peptides, for aligning the three tryptic fragments, a chymotryptic digestion of the whole peptide was performed, and the resulting fragments were separated by paper electrophoresis. Of the several peptides isolated, C-VI was unique having the sequence: Gly-Gly-Arg-Arg. This peptide apparently constituted the overlaps of the tryptic fragments, T-II and T-III (free arginine), and indicated the following alignment: T-II ⟶ T-III ⟶ T-I.

T-I fragment

Chymotryptic digestion of T-I yielded two fragments, T-I-C-1 and T-I-C-2, in which the former constituted the N-terminal portion of T-I, since the N-terminal alanine and amino acid composition were identical to the N-terminal portion of the fragment, T-I (Table 2). Moreover, the amino acid composition of T-I-C-2 was similar to the C-terminal portion of T-I.

Thermolytic digestion of T-I yielded five fragments, and

the Edman-dansyl analysis of T-I-TL-3 and T-I-TL-5 established the N-terminal sequence of T-I up to 16 residues (Table 2). The fragments, T-I-TL-4 and T-I-TL-2 had the C-terminal sequence (Val-Glu) and N-terminal sequence (Gln-Gly) of the above two chymotryptic fragments, T-I-C-1 and T-I-C-2, respectively. The final thermolytic fragment, T-I-TL-1, must have constituted the C-terminal sequence of T-I, because it contained C-terminal arginine. These results provided all the alignments of the thermolytic and chymotryptic fragments derived from T-I.

The complete amino acid sequence of the whole peptide is shown in Fig. 1.

DISCUSSION

Previously, we reported that the activation of bovine Factor XIII by thrombin must involve a limited proteolysis of the arginyl-glycyl bond located in the N-terminal region of the parent molecule, liberating a small peptide with a masked N-terminal residue (6). The present work establishes the amino acid sequence of the entire peptide. In the digestion with trypsin, the two theoretically expected trypsin sensitive peptide bonds were hydrolyzed and the three fragments were isolated in high yields (41 to 90 %). Furthermore, the sum of the amino acids in the tryptic fragments was equal to the composition of the original peptide (Table 1). The sequence analysis for the N-terminal T-II fragment was troublesome because of its masked N-terminal end. However, this problem was overcome by using the acylamino acid releasing enzyme recently purified from rat liver (9). This enzyme cleaved the N-acetylserylglutamyl bond of fragment T-II-C-1, yielding free N-acetylserine and the remaining peptide now reactive to dansyl-chloride. Thus, the above enzymatic method made it possible to determine the total sequence of T-II.

A comparison of the entire sequences of the human and bovine peptides reveals 5 amino acid replacements, Ser-3 to Thr; Gly-5 to Arg; Ile-14 to Val; Thr-18 to Asn and Pro-26 to Leu. Another notable difference is the deletion of Leu-34 in the human peptide (Fig. 1).

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