Amino Acid Sequence Studies on Factor XIII and the Peptide Released During Its Activation by Thrombin†

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ABSTRACT: The amino acid sequence of the 36-residue peptide released during the activation of human plasma factor XIII by thrombin has been determined. The corresponding peptide from bovine material is made up of 37 amino acids; besides the additional residue, 5 amino acid interchanges are evident upon comparison with the human peptide. The amino-terminal sequences of the two nonidentical polypeptide chains comprising plasma factor XIII were also studied, before and after activation. In both the human and bovine, arginylglycine bonds in one of the chains (a chain) are cleaved by thrombin during the activation process. Before activation, the amino terminal of that chain is blocked, and studies on the released peptides have shown that the block is due to N-acetylation. The activation peptide released from human platelet factor XIII has also been characterized, and it appears to be identical with the peptide released from the plasma zymogen. Moreover, the amino-terminal sequence of the activated platelet factor was also indistinguishable from the corresponding region of the plasma enzyme, all of these observations being consistent with the idea that platelet factor XIII is identical with the a chain of the circulating plasma factor.

Factor XIII is the precursor of a transamidase-type enzyme which stabilizes vertebrate fibrin by the introduction of ε-(γ-glutamyl)lysine cross-links.[1] The calcium-dependent enzyme, which is activated by thrombin (Buluk *et al.*, 1961; Lorand and Konishi, 1964), exists in two forms, one found in plasma and another in blood platelets and certain other tissues (Bohn, 1970). The plasma factor is composed of two nonidentical chains designated a and b (Schwartz *et al.*, 1971; Takagi and Konishi, 1972) and has a molecular weight of about 320,000 (Loewy *et al.*, 1961a; Schwartz *et al.*, 1973; Takagi and Konishi, 1972). The molecular weights of the a and b chains as determined on sodium dodecylsulfate gels vary somewhat with species, but are usually similar and between 75,000 and 85,000, corresponding to a structural arrangement of a_2b_2. The subunits are not held together by interchain disulfide bonds. The platelet factor is made up only of dimerized a chains, a native molecular weight of about 180,000 having been reported (Bohn, 1970). On sodium dodecyl sulfate gels the a chains of human platelets and plasma are reportedly indistinguishable (Schwartz *et al.*, 1971). In the case of both the plasma and platelet factors, treatment with thrombin results in a diminution of the a-chain molecular weight (Schwartz *et al.*, 1971), indicating that peptide material has been removed. Calcium ions do not have to be present for the thrombin-mediated event, but the transamidase activity does require their presence (Lorand and Konishi, 1964; Schwartz *et al.*, 1973; Takagi and Doolittle, 1973).

This report deals with an investigation of the thrombin-catalyzed activation of factor XIII from human and bovine plasma and human platelets. We have established that in all three cases thrombin treatment results in the removal of a single polypeptide from the amino terminus of the a chain. The nature of the linkage split has been determined, as well as the amino acid sequences of the amino-terminal regions of the b and activated a chains. The entire amino acid sequence of the activation peptide (n = 36 residues) released from human factor XIII has been determined. The sequence of the corresponding peptide from the bovine factor (n = 37 residues) has also been studied in detail and the positions of five amino acid replacements established. Finally, the amino acid compositions of the tryptic peptides derived from the (human) platelet activation peptide have been determined, no differences being found upon comparison with the (human) plasma peptide.

Experimental Section

Materials

Human plasma and platelets were obtained from the San Diego Blood Bank. Bovine (domestic cattle) blood was collected at local slaughter houses, trisodium citrate being used as an anticoagulant. A bovine fraction obtained by ammonium sulfate precipitation and containing factor XIII was kindly provided by Drs. K. Fujikawa and E. Davie of the University of Washington, Seattle. Thrombin (Parke-Davis, Topical) was purified according to the method of Baughman and Waugh (1967). Tos-PheCH$_2$Cl-trypsin,[2] α-chymotrypsin, pepsin, carboxypeptidase A, and carboxypeptidase B were purchased from Worthington Biochemicals. Pronase was purchased from Calbiochem, and thermolysin was a gift from Dr. H. Matsubara. Pyrrolidonecarboxylic acid peptidase was purified from a strain of *Pseudomonas fluorescens* (Doolittle, 1972).

Thioacetylthioglycolic acid (TATG) was synthesized according to the procedure of Jensen and Pedersen (1961). Phenyl isothiocyanate (PhSCN) and dimethylallylamine were purchased from Eastman and were distilled before use. 5-Dimethylaminonaphthalene-1-sulfonyl (Dns) chloride was obtained from Pierce Chemicals.

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[1] A wide-ranging collection of papers on fibrin stabilization by factor XIII is available in *Ann. N. Y. Acad. Sci. 202*, 1–348 (1972).

[2] Abbreviations used are: Tos-PheCH$_2$Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; TATG, thioacetylthioglycolic acid; PhSCN, phenyl isothiocyanate; Dns, 5-dimethylaminonaphthalene-1-sulfonyl.

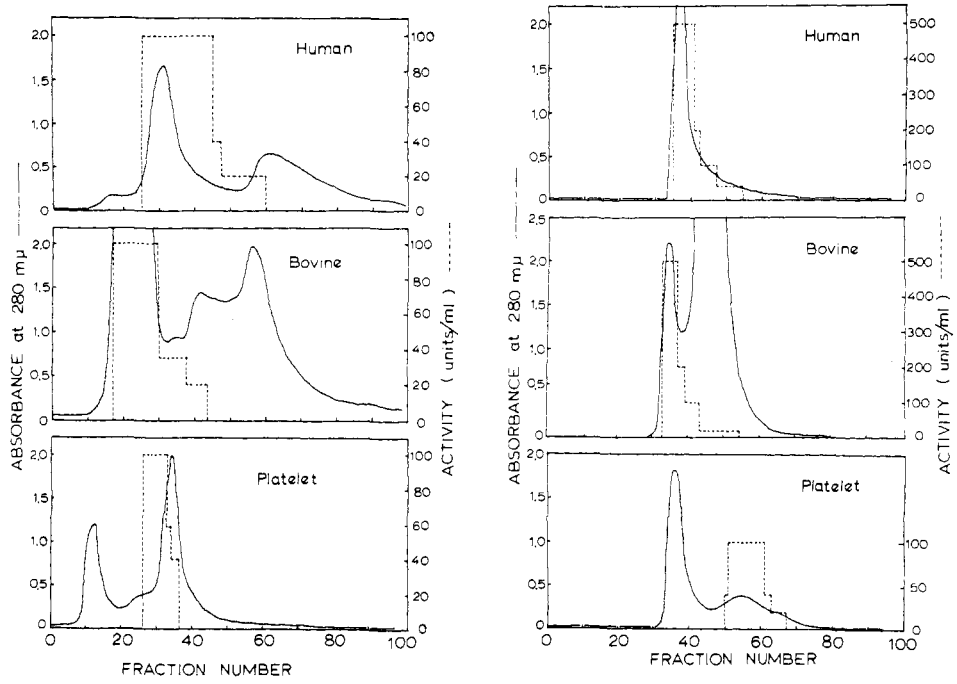

FIGURE 1: Purification of factor XIII from human plasma, bovine plasma, and human platelets. The graphs on the left side are the elution profiles obtained from DE-52 (2.3 × 23 cm). Columns were equilibrated with 0.05 M Tris (pH 7.5), containing 0.001 M EDTA, and elution obtained with a limiting 0.3 M NaCl gradient. Fraction size = 10.0 ml; gradient = 500 ml of starting buffer + 500 ml of limit buffer. The profiles on the right represent elutions from a Bio-Gel A1.5m column (2.5 × 90 cm) which was equilibrated with 0.05 M Tris (pH 7.5), containing 0.1 M NaCl and 0.001 M EDTA; fraction size = *ca.* 5 ml.

Methods

Purification of Factor XIII from Various Sources. Factor XIII was purified and assayed according to previously described procedures (Takagi and Konishi, 1972). In the case of the plasma factor, the method involves a series of ammonium sulfate precipitations (usually starting with about 10 l. of plasma) followed by a heat precipitation step (Loewy *et al.*, 1961b). The supernatant after the heat treatment (3 min at 56°) was concentrated by ammonium sulfate precipitation, redissolved in 0.05 M Tris (pH 7.5) containing 0.001 M EDTA, and then dialyzed against that buffer before chromatography on DE-52 (Takagi and Konishi, 1972). The DE-52 fractions containing factor XIII activity were pooled, precipitated with ammonium sulfate, redissolved, and gel filtered on Bio-Gel A-1.5m (Takagi and Konishi, 1972). Active fractions were precipitated with ammonium sulfate and then redissolved in 0.05 M Tris (pH 7.5) containing 0.1 M NaCl. The solutions were dialyzed against the same buffer prior to activation by thrombin. Preparations at this stage gave two bands upon sodium dodecyl sulfate gel electrophoresis (Weber and Osborne, 1969) in both the presence of absence of reducing agent.

Human platelet factor XIII was isolated from platelets which were first washed three times with 0.15 M NaCl and then stored frozen as pellets until used. The pellets were suspended in minimal amounts of 0.05 M Tris (pH 7.5) containing 0.001 M EDTA and then subjected to sonication by two 30-sec blasts with a Branson sonifier. The sonicate was centrifuged and the supernatant fluid was dialyzed before chromatography on DE-52 (Figure 1). Active fractions were pooled and concentrated and then applied to Bio-Gel in the same manner as the plasma preparations. In this case, however, the active peak was considerably more retarded than in the case of the plasma enzymes (Figure 1), reflecting its lower molecular weight. Only one major band appeared on sodium dodecyl sulfate gel electrophoresis, corresponding to the larger of the two components observed in the human plasma preparation.

Peptide Isolation. After the original separation of activation peptide material by gel filtration on Sephadex G-50, all separations and further purification were accomplished by low-voltage electrophoresis at pH 2.0 (8% acetic acid–2% formic, v/v), pH 6.5 (pyridine–acetic acid–water, 100:4:900), or in 0.1 M pyridine buffers adjusted to pH 3.5, 4.1, or 5.0 with acetic acid. Guide strips were stained with either ninhydrin or the arginine-detecting stain of Yamada and Itano (1966). The chlorine stain for ninhydrin-negative materials was used according to the method of Reindel and Hoppe (1954), and tryptophan was tested for using an Ehrlich stain (Smith, 1960).

Enzymic Hydrolysis and Partial Acid Hydrolysis. Trypsin and chymotrypsin digestions were conducted in 0.1 M ammonium bicarbonate (pH 7.9–8.0) at 37° for 4 hr. Thermolysin digestions were carried out at 37° for 30–40 min in a 0.01 M Tris buffer (pH 8.1), containing 0.0003 M $CaCl_2$. Pepsin digestion was accomplished in four hours at 37° in 0.01 N HCl. Pronase digestions were at room temperature overnight and carboxypeptidase digestions at 37° for varying times. In all cases the enzyme concentrations were approximately 0.1 mg/ml. Partial acid hydrolysis was attained with 0.03 N HCl in sealed tubes at 105° for 16–17 hr.

Amino acid analysis was performed on a Spinco Model 117 analyzer employing a single-column, three-buffer, system as specified by the manufacturers. Peptides were hydrolyzed with 5.7 N HCl at 105° for 24 hr.

Sequential Degradations. Amino acid sequences were determined on individual peptides using the Dns-PhSCN stepwise degradation procedure as outlined by Gray (1967) and Hartley (1970). Identification of Dns-amino acids was accomplished on polyamide thin-layer sheets (Cheng Chin Trading Co.,

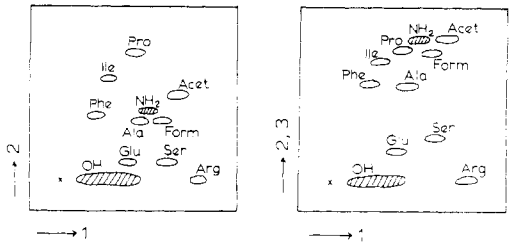

FIGURE 2: Positions of acetyl-Dns-hydrazide ("Acet") and formyl-Dns-hydrazide ("Form") on polyamide sheets using the standard 1:2:3 solvent system of Woods and Wang (1967). The relative positions of some Dns-amino acids, Dns-hydroxide ("OH"), and Dns-amine ("NH_2") are also shown.

Ltd.) using the solvent systems described by Woods and Wang (1967).

Determination of N-Acetyl Groups. The presence of acetylated amino groups was established by Dns treatment at pH 3 of the products of hydrazinolysis, essentially according to the method of Schmer and Kreil (1969), except that ethyl acetate was used instead of chloroform or ether for extracting Dns derivatives from the coupling system. *N*-Acetyltyrosine, *N*-acetylhistidine, and *N*-formylglutamic acid (the generous gift of Dr. M. Friedkin, Department of Biology, University of California, San Diego) were used as reference substances to check the procedures. Dns-acetyl hydrazide and Dns-formyl hydrazide were found to be well separated by the standard thin-layer system for Dns-amino acids (Figure 2).

End-Group Determination. The amino-terminal sequences of the various factor XIII preparations were determined using the thioacetylation procedure described by Mross and Doolittle (1971). The advantage of this particular stepwise degradation procedure is that the free amino acid is readily regenerated and can be quantified on a standard amino acid analyzer. This is particularly useful when more than one polypeptide chain is being examined simultaneously. The protein to be studied (5–20 mg) was dissolved in 1.0 ml of 6 M guanidine hydrochloride–0.1 M Tris buffer (pH 9.5). An equal volume of pyridine containing 20 mg of TATG was added and the pH was adjusted to 9.4–9.6. Coupling was allowed to proceed at 40° for 60 min. The protein was precipitated by the addition of acetone (6 ml), and the precipitate was washed three times with 90% aqueous acetone before freeze-drying over P_2O_5. After thorough drying, cleavage was effected by the addition of 200 μl of trifluoroacetic acid and incubation at 40° for 20 min. The cleaved derivatives were extracted with 1,2-dichloroethane twice (*ca.* 1.5 ml each time), the extractants being transferred directly to a hydrolysis tube containing 0.2 ml of 5.7 N HCl. After mixing, the organic phase was removed by a stream of nitrogen. Tubes were then sealed under reduced pressure and placed either at 130° for 4 hr or 105° for 16 hr. The acid was evaporated over NaOH pellets before the residues were dissolved in standard dilutor buffer and applied to the amino acid analyzer.

Results

Isolation of the Activation Peptide. Factor XIII preparations (5 mg/ml) were treated with thrombin (final concentration 10 NIH units/ml), the mixture being incubated at room temperature for 2–2.5 hr. Sodium dodecyl sulfate gel electrophoresis revealed that the conversion of the a chain to its shorter (active) form was virtually complete within 5 min. In most experiments 60–100 mg of factor XIII was used. The reactions were conducted both in the presence and absence of 0.02 M $CaCl_2$. In the presence of calcium ions, all the factor XIII preparations precipitated as a result of self-induced cross-linking (Bohn, 1972; Takagi and Doolittle, 1973). At the end of the digestion an equal volume of cold absolute ethanol was added in order to precipitate the bulk of the protein. After 30 min, the precipitate was removed by centrifugation and washed twice. The supernatants were concentrated on a rotary evaporator at 38°. The residue was dissolved in a small volume of 0.1 M ammonium bicarbonate and passed over a Sephadex G-50 column (Figure 3). Peak II gave a single ninhydrin-negative, arginine-positive band upon electrophoresis at pH 2.

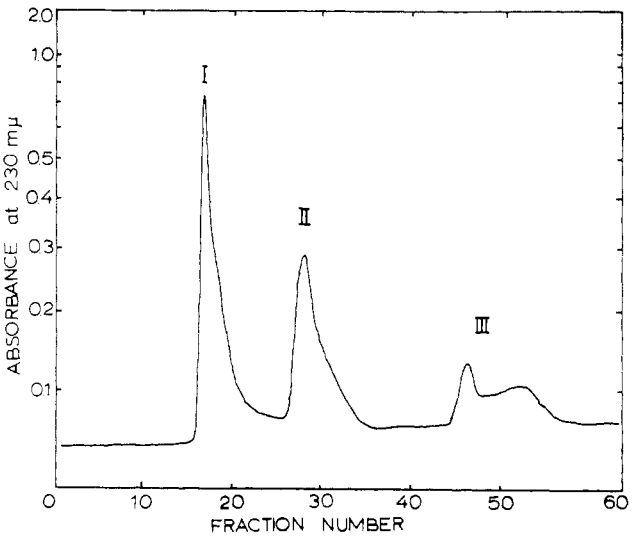

FIGURE 3: Purification of the peptide released from human plasma factor XIII upon thrombin activation by gel filtration on Sephadex G-50 (2.5 × 92 cm) equilibrated with 0.1 M ammonium bicarbonate (flow rate 40 ml/hr; fraction size 5 ml). Peak I was contaminating protein, peak II the activation peptide, and peak III mainly salt.

Amino Acid Compositions. The amino acid compositions of the activation peptide isolated from each of the three different systems (human and bovine plasma and human platelet enzyme) are listed in Table I. The human plasma and platelet peptides both contained the same 36 amino acids, whereas the bovine plasma activation peptide is comprised of 37 residues. Several differences between the human and bovine material were apparent at this stage, the human peptides containing additional residues of aspartic acid, valine, and arginine, but less serine, proline, glycine, and isoleucine.

The various activation peptides were digested with trypsin and the fragments separated by low-voltage electrophoresis at pH 4.1 and 2.0. At this stage the amino acid composition data indicated that there were no differences between the two human activation peptides (plasma and platelet), but that there were at least four differences between human and bovine (Table II). Stepwise sequence procedures, as well as fragmentation by other enzymes, ultimately showed that there were five amino acid replacements between bovine and human, in addition to the extra residue found in the bovine peptide.

Sequence Summaries. The data on each of the isolated peptide fragments derived from trypsin (T), chymotrypsin (C), thermolysin (Th), pepsin (P), and partial acid hydrolysis (A) are tabulated in the following paragraphs. A summary of this information is also depicted in Figures 4 and 5 for the human plasma and bovine plasma activation peptides, respectively. The human plasma activation peptide fragments are designated H, the bovine peptide B, and the human platelet pep-

TABLE I: Amino Acid Composition of Factor XIII Activation Peptides.[a]

	Bovine Plasma	Human Plasma	Human Platelet
Aspartic acid	4.3 (4)	5.2 (5)	5.0 (5)
Threonine	2.9 (3)	2.8 (3)	2.7 (3)
Serine	3.7 (4)	2.9 (3)	2.7 (3)
Glutamic acid	4.0 (4)	4.2 (4)	4.0 (4)
Proline	4.6 (5)	3.9 (4)	4.1 (4)
Glycine	4.3 (4)	3.1 (3)	2.8 (3)
Alanine	4.1 (4)	4.0 (4)	4.0 (4)
Valine	1.9 (2)	3.0 (3)	3.3 (3)
Isoleucine	1.0 (1)		
Leucine	1.9 (2)	2.3 (2)	2.3 (2)
Phenylalanine	1.0 (1)	0.9 (1)	1.3 (1)
Arginine	3.1 (3)	3.8 (4)	3.7 (4)
Total	36.8 (37)	36.1 (36)	35.9 (36)

[a] Values are expressed as molar ratios, the nearest integer appearing in parentheses. In the case of the human plasma activation peptide, the results are the average of six separate determinations. In the case of the bovine, two separate analyses have been averaged; our results are in agreement with the composition recently reported by Mikuni *et al.* (1973). Only enough material was available for a single analysis of the human platelet peptide.

tide X. Compositions of all peptides were determined quantitatively on an automatic amino acid analyzer. All stepwise sequences were determined by the Dns-PhSCN stepwise procedure. Assignments of amides (glutamine and asparagine) were made on the basis of electrophoretic mobilities.

Human Plasma Activation Peptide Fragments

HT-1 ($n = 5$): *N*-Ac-(Ser,Glu,Thr)Ser-Arg.

HT-2 ($n = 24$): Ala-Val-Pro-Pro-Asx-Asx-Ser-Asx-Ala-Ala-Glx-Asx(Asx,Leu,Pro,Thr,Val,Glx,Leu,Glx,Gly,Val,Pro)-Arg.

HT-3 ($n = 25$): Arg-Ala-Val(Pro,Pro)Asx-Asx(Ser,Asx,Ala,-Ala,Glx,Asx,Asx,Leu,Pro,Thr,Val,Glx,Leu,Glx,Gly,Val,-Pro)Arg.

HT-4 ($n = 6$): Thr(Ala,Phe,Gly,Gly)Arg.

HT-5 ($n = 1$): Arg.

HT-2-Th-1 ($n = 8$): Ala-Ala-Glu-Asp-Asp(Leu,Pro,Thr).

HT-2-Th-2 ($n = 2$): Val-Glu.

HT-2-Th-3a ($n = 8$): Ala-Val(Pro,Pro,Asn,Asn,Ser,Asn).

HT-2-Th-3b ($n = 3$): Leu-Gln-Gly.

HT-2-C-3 ($n = 5$): Gln-Gly-Val-Pro-Arg.

HT-4-C-1 ($n = 3$): Thr-Ala-Phe.

HT-4-C-2 ($n = 3$): Gly-Gly-Arg.

HP-1 ($n = 7$): Asp-Asp-Leu-Pro-Thr-Val-Glu.

HT-1-A-1 ($n = 5$): Ser-Glu-Thr(Ser)Arg.

Bovine Plasma Activation Peptide Fragments

BT-1 ($n = 11$): *N*-Ac(Ser,Glu,Thr,Ser,Gly,Ser,Ala,Phe,Gly,-Gly)Arg.

BT-2 ($n = 25$): 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.

BT-3 ($n = 1$): Arg.

BCO and BT-1-CO ($n = 8$): N-Ac(Ser,Glx,Thr,Ser,Gly,Ser,-Ala)Phe.

BC-1 ($n = 13$): (Ala,Ala,Glx,Asx,Asx,Pro,Pro,Thr,Val,Glx,-Leu).

BC-2 ($n = 23$): (Gly,Gly,Arg,Arg,Ala,Ile,Pro,Pro,Asx,Thr,-Ser,Asx,Ala,Ala,Glx,Asx,Asx,Pro,Pro,Thr,Val,Glx,Leu).

BC-3 ($n = 6$): (Gln,Gly,Leu,Val,Pro,Arg).

BT-1-C-1 ($n = 3$): Gly-Gly-Arg.

BT-2-Th-1 ($n = 8$): Ala-Ala-Glu(Asp,Asp,Pro,Pro,Thr).

BT-2-Th-2 ($n = 2$): Val-Glu.

BT-2-Th-3a ($n = 8$): Ala-Ile-Pro-Pro-Asn-Thr-Ser-Asn.

BT-2-Th-3b ($n = 3$): Leu-Gln-Gly.

BT-2-Th-4 ($n = 4$): Leu-Val-Pro-Arg.

Human Platelet Activation Peptide Fragments

TABLE II: Amino Acid Compositions of Tryptic Fragments Derived from Human and Bovine Plasma and Human Platelet Factor XIII Activation Peptide.[a]

	Bovine Plasma XIII			Human Plasma XIII					Human Platelet XIII			
	T-1	T-2	T-3	T-1	T-2	T-3	T-4	T-5	T-1	T-2	T-4	T-5
Aspartic acid		3.9 (4)			5.1 (5)	5.2 (5)				5.1 (5)		
Threonine	1.1 (1)	1.9 (2)		1.0 (1)	1.1 (1)	1.0 (1)	0.9 (1)		0.8 (1)	0.9 (1)	0.8 (1)	
Serine	2.6 (3)	0.9 (1)		1.8 (2)	1.1 (1)	1.1 (1)			1.9 (2)	0.9 (1)		
Glutamic acid	1.4 (1)	2.8 (3)		1.2 (1)	3.0 (3)	2.9 (3)			1.2 (1)	2.9 (3)		
Proline		5.1 (5)			4.4 (4)	4.3 (4)				3.5 (4)		
Glycine	2.8 (3)	1.8 (2)			0.9 (1)	1.3 (1)	2.2 (2)			1.2 (1)	2.1 (2)	
Alanine	1.1 (1)	2.8 (3)			2.7 (3)	2.6 (3)	0.9 (1)			3.0 (3)	0.9 (1)	
Valine		1.8 (2)			2.7 (3)	3.1 (3)				3.0 (3)		
Isoleucine		0.9 (1)										
Leucine		2.1 (2)			2.1 (2)	2.3 (2)				2.4 (2)		
Phenylalanine	0.9 (1)						1.1 (1)				1.0 (1)	
Arginine	0.7 (1)	0.9 (1)	1.0 (1)	0.8 (1)	0.8 (1)	1.5 (2)	0.9 (1)	1.0 (1)	0.9 (1)	0.9 (1)	1.1 (1)	1.0 (1)
Total residues	11	25	1	5	24	25	6	1	5	24	6	1
Approximate yield (%)	69	45	35	47	29	10	29	7	56	36	46	26
Position in sequence	1–11	13–37	12	1–5	13–36	12–36	6–11	12	1–5	13–36	6–11	12

[a] Values are expressed as molar ratios, the nearest integer appearing in parentheses. The nomenclature for human platelet subpeptides was kept the same as for the human plasma type, even though the low-yield overlap peptide, T-3, was not isolated.

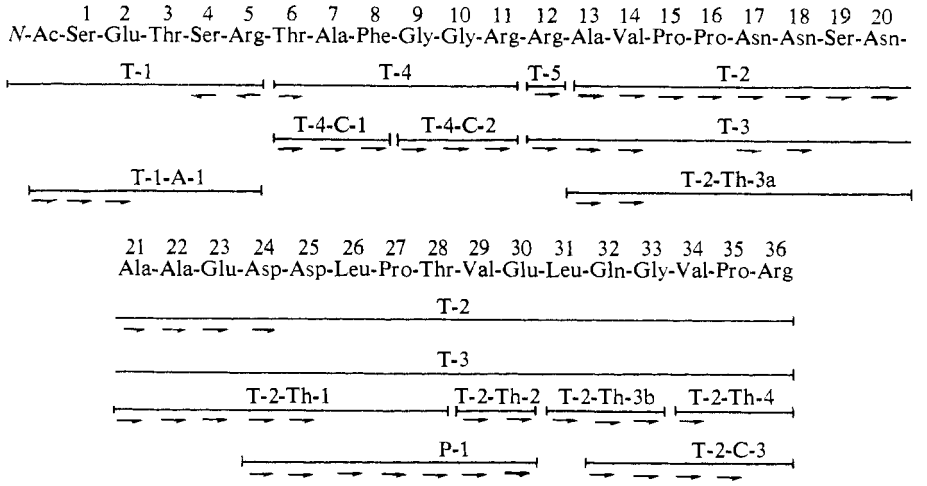

FIGURE 4: Summary of data used in establishing the amino acid sequence of the peptide released from human plasma factor XIII by thrombin: T = trypsin; Th = thermolysin; C = chymotrypsin; P = pepsin; A = partial acid hydrolysis; → = Dns-PhSCN; ← = carboxypeptidase A or B.

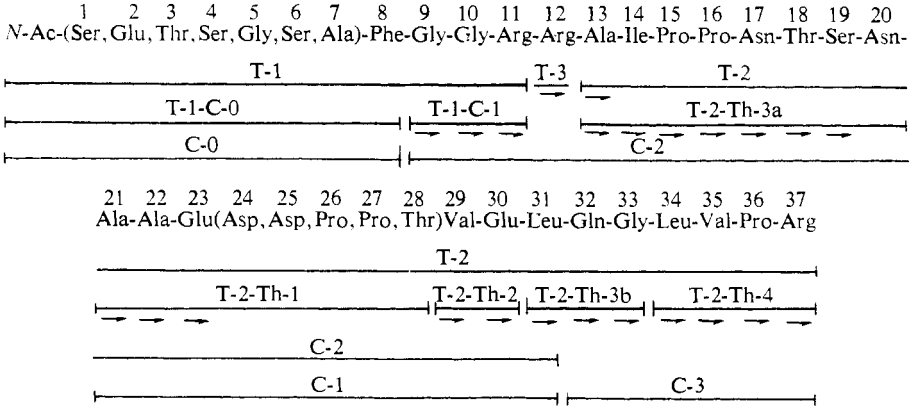

FIGURE 5: Summary of data used in characterization of peptide released from bovine plasma factor XIII. The abbreviations used are the same as those given in Figure 4.

XT-1 (n = 5): *N*-Ac(Ser,Glu,Thr,Ser)Arg.

XT-2 (n = 24): Ala(Val,Pro,Pro,Asx,Asx,Ser,Asx,Ala,Ala,-Glx,Asx,Asx,Leu,Pro,Thr,Val,Glx,Leu,Glx,Gly,Val,Pro)-Arg.

XT-4 (n = 6): Thr-Ala-Phe-Gly-Gly-Arg.

XT-5 (n = 1): Arg.

Nature of the Blocked Amino Terminals. All three of the activation peptides were ninhydrin negative and failed to yield Dns-amino acids upon derivatization. Digestion of the various activation peptides and/or their terminal fragments with pyrrolidonecarboxylyl peptidase failed to change the peptide mobilities or expose new endgroups. Treatment of peptides HT-1, BT-1, and XT-1 with the hydrazine-Dns procedure clearly indicated that the blocks were due to N-acetylation.

Amino-Terminal Sequence Studies. Factor XIII preparations from human and bovine plasma were subjected to a series of stepwise degradations by the TATG procedure, before and after activation by thrombin. Because the unactivated enzyme has its a chain blocked, only the b-chain sequence is obtained before thrombin treatment. After activation, the newly exposed amino terminal of the a chain is also susceptible to degradation, and two sets of sequences are determined simultaneously. The new sequence is readily deduced by subtracting the residues found on the unactivated preparation (Table III). The first six residues of the single-chained activated human platelet factor XIII were also determined.

The b chains of the human and bovine plasma enzymes both begin with the sequence Glu-Glx-Lys-Pro (Figure 6), the designation of glutamic acid as the endgroup (rather than glutamine) being a surmize based on the logic that amino-terminal glutamine would have been largely converted into pyrrolidonecarboxylic acid. After activation by thrombin, the a-chain sequences could also be studied. In all three cases the newly exposed end group was glycine. Beyond that, the two human factors (plasma and platelet) were found to have identical sequences for their first six residues (Figure 6). In contrast, the activated a chain from bovine plasma material was different from the human type in at least two of its first six positions (Table III and Figure 6).

Discussion

One of our primary objectives in characterizing factor XIII was to compare it with fibrinogen, another protein which is a substrate for thrombin. We were especially interested in this comparison after finding that activated factor XIII can cross-link itself, as well as fibrin (Takagi and Doolittle, 1973). Al-

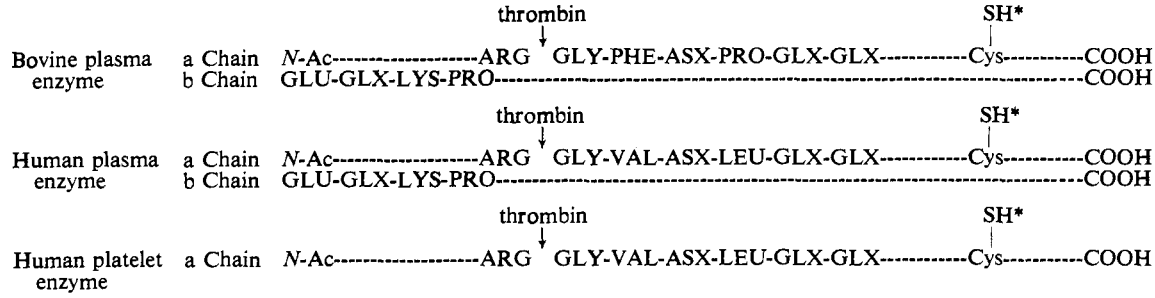

FIGURE 6: Schematic depiction of thrombin action on factor XIII from human and bovine plasma and human platelets. The platelet enzyme is composed only of a chains, whereas the plasma enzymes have accessory b chains also. The active SH group involved in factor XIII transamidase action is indicated for illustrative purposes; its exact location in the a chain is not yet determined.

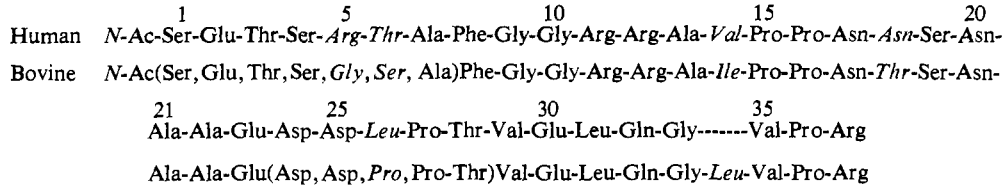

FIGURE 7: Comparison of peptides released from human and bovine plasma factor XIII. The five amino acid differences and the one "gap" are in italic type.

TABLE III: Amino Acids Released during Stepwise Degradation[a] of Human and Bovine Plasma Factor XIII Preparations before and after Activation by Thrombin.

Step	Human Plasma			Bovine Plasma			Human Platelet
	Before	After		Before	After		After
1	Glu (31.7)	Glu (31.0)	Gly (30.0)	Glu (36.8)	Glu (29.0)	Gly (27.0)	Gly (16.8)
2	Glx (41.3)	Glx (28.4)	Val (10.7)	Glx (17.2)	Glx (14.5)	Phe (16.4)	Val (24.0)
3	Lys (26.5)	Lys (14.8)	Asx (11.4)	Lys (12.0)	Lys (11.0)	Asx (16.9)	Asx (10.6)
4	Pro (12.9)	Pro (9.4)	Leu (12.6)	Pro (13.4)	Pro (22/2)	Pro (22/2)	Leu (7.8)
5			Glx (17.4)			Glx (16.0)	Glx (15.5)
6			Glx (16.2)			Glx (18.7)	Glx (11.7)

[a] Stepwise degradation by the thioacetylation procedure using thioacetylthioglycollic acid (TATG) followed by acid hydrolysis of the extracted derivative. Values in parentheses are nanomoles actually recovered on analyzer; in each case the degradations were started on approximately 40–60 nmol of factor XIII.

though both fibrinogen and factor XIII from plasma are large proteins, they have fundamentally different chain arrangements, fibrinogen having two sets of three nonidentical chains corresponding to an $\alpha_2\beta_2\gamma_2$ arrangement, all of the chains being bound together by disulfide bonds. In factor XIII, the two sets of a and b chains are tightly associated but not covalently attached. Still, there was the remote possibility that these two proteins might have arisen from a common ancestor, and a comparison of the peptides released by thrombin in the two situations seemed warranted.

As it turned out, in both cases thrombin cleaves arginylglycine bonds and releases peptide material from the amino-terminal region of a large polypeptide chain. On the other hand, there is no apparent resemblance between the sequences of the fibrinopeptides—either A or B—with the activation peptide released from human or bovine factor XIII. The fibrinopeptides are very changeable, however, and it is not impossible that any similarities of structure might have been completely blurred during the passage of evolutionary time. On the other hand, the activation peptides released from factor XIII appear to be significantly less variable than fibrinopeptides. For example, the bovine and human fibrinopeptides A and B differ in 17 of 30 comparable residues, but the activation peptides from these two species only differ in six positions of 37 compared (Figure 7). Nor is their any obvious homology on the other side of the bond cleaved by thrombin (Figure 8). The only discernible feature common to both appears to be the arginylglycine linkage itself.

All of our data on the platelet activation peptide and the amino-terminal sequence of the platelet enzyme confirm earlier suppositions that the platelet enzyme is identical with the plasma factor XIII a chain. Thus, persons defective in plasma factor have also been found to lack the platelet enzyme (Loewy, 1970). Moreover, Bohn *et al.* (1973), using an immunochemical approach, have found that such patients actually have material corresponding to b chains (subunit S in their terminology), and that this material can be combined with the platelet enzyme from normal persons to yield a normal-type plasma factor. Schwartz *et al.* (1973) have found that the platelet enzyme and the plasma factor a chain are indistinguishable on sodium dodecyl sulfate gels and by overall amino acid composition, and they were able to combine the

Factor XIII ..Val-Pro-ARG ↓ GLY-$\frac{Val}{Phe}$-Asx-$\frac{Leu}{Pro}$-Glx-Glx..

Fibrinogen Aα ..Gly-Val-ARG ↓ GLY-Pro-Arg-Val-Val-Glu..

Fibrinogen Bβ ..Ser-Ala-ARG ↓ GLY-His-Arg-Pro-Leu-Asp..

FIGURE 8: Comparison of thrombin-sensitive sequences in factor XIII (human and bovine composite) with those of human fibrinogen (Blombäck *et al.*, 1968).

plasma factor b chain with the platelet enzyme to yield a product equivalent to the native plasma factor. Bohn *et al.* (1972) has previously reported a similar experiment recombining b chains (subunit S) with a placental enzyme which they showed was immunochemically identical with the platelet enzyme. It has been suggested that the role of the b chain (subunit S) is to "stabilize" the circulating enzyme or that it acts as a transport protein (Bohn *et al.*, 1972, 1973).

The fact that a skein of 42 amino acids (36 in the activation peptide and 6 on the parent chain) has been examined (although not fully sequenced) and no evidence of any amino acid replacement found makes it very likely that the platelet enzyme and the plasma factor a chain are identical gene products. What remains mysterious is how and where the a and b chains of the plasma factor are combined and/or dissociated. Are a chains made in the megakaryocyte–platelet system and then combined with circulating b chains? Or are a chains made elsewhere and sequestered away from b chains by platelets and other tissues, such as the placenta (Bohn *et al.*, 1972)? A previous report (McDonagh *et al.*, 1969) indicated the plasma factor was made in liver, whereas the platelet factor is made in megakaryocytes. The fact that the isolated platelet enzyme is readily soluble in physiological buffers argues against a role for the b chain as a solubilizing agent. Finally, it will also be of interest to learn if the a chain of the plasma factor—and therefore the platelet–placental enzyme—is homologous to the transamidases of other tissues, none of which has been found to exist in a precursor form (Folk and Chung, 1973). Further sequence studies on these enzymes ought to answer this question in the near future.

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