

Primary structure of blood coagulation factor XIIIa (fibrinolygase, transglutaminase) from human placenta

[amino acid sequence/clot-stabilizing enzyme/ ϵ -(γ -glutamyl)lysine crosslinks/calcium-binding protein/active thiol site]

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ABSTRACT We have determined the primary structure of human placental factor XIIIa, an enzyme [fibrinolygase, transglutaminase, fibrin-stabilizing factor, EC 2.3.2.13 (protein-glutamine:amine γ -glutamyltransferase)] that forms intermolecular isopeptide bonds between fibrin molecules as the last step in blood coagulation. Placental factor XIIIa is an unglycosylated polypeptide chain of 730 amino acid residues ($M_r = 83,005$) that appears to be identical to the *a* subunit of the plasma zymogen factor XIII. Ca^{2+} -dependent activation of factor XIIIa by thrombin removes a blocked amino-terminal peptide and unmasks a reactive thiol group at Cys-314. A second specific cleavage after Lys-513 by thrombin inactivates factor XIIIa and produces an amino-terminal 56-kDa fragment and a 24-kDa fragment. The amino acid sequence of factor XIIIa is unique and does not exhibit internal homology, but its active center is similar to that of the thiol proteases. The probable Ca^{2+} -binding site of factor XIIIa has been identified by homology to the high-affinity sites in calmodulins. Knowledge of the primary structure of factor XIIIa will aid elucidation of the mechanism of its enzymatic action and that of the many tissue transglutaminases of which it is the prototype. This will also facilitate production of factor XIIIa by recombinant DNA technology for use in treatment of congenital factor XIII deficiencies and in the postoperative healing of wounds.

Factor XIII_a [FXIII_a; fibrinolygase, fibrin-stabilizing factor, EC 2.3.2.13 (protein-glutamine:amine γ -glutamyltransferase)] is the last enzyme generated in the blood coagulation cascade (1, 2). It is a transglutaminase or transamidating enzyme that forms intermolecular γ -glutamyl- ϵ -lysine crosslinks between fibrin molecules, thereby stabilizing the fibrin clot mechanically and also conferring resistance to proteolysis. FXIII_a has a cysteine thiol active site; however, unlike thiol proteases such as cathepsins B and H that cleave peptide bonds, FXIII_a forms intermolecular isopeptide bridges in fibrin. Other substrates for FXIII_a include coagulation factor V, α_2 -macroglobulin, platelet myosin, actin, and fibronectin. Thus, in addition to its essential role in blood clotting, FXIII_a may function to stabilize cell surface molecules and membranes. FXIII_a is the prototype of a class of Ca^{2+} -dependent transglutaminases with thiol active centers that are widespread in animal tissues and have been associated with cell proliferation, embryonic development, and growth (3–5). However, none except FXIII_a has been purified and characterized, and until now little has been published about the structure of FXIII_a except the sequence of the amino-terminal activation peptide of the human (6) and bovine (7) plasma FXIII_a. We report here the primary structure of human placental FXIII_a, including the sequence at the active site, and the sites of activation and inactivation by thrombin and the probable Ca^{2+} -binding site.

FXIII_a is formed by a two-step activation of the proenzyme FXIII, which is present in plasma as a tetrameric noncovalent complex ($M_r \approx 340,000$) composed of two catalytic *a* chains ($M_r = 83,000$) and two noncatalytic *b* chains ($M_r = 85,000$) with the formula a_2b_2 (1–3). However, platelet and placental FXIII lack *b* chains and have the subunit composition a_2 . The *b* chain has no role for FXIII_a activity, but it is thought to have a protective function for circulating plasma FXIII. Although composed only of *a* chains, the platelet and placental enzymes (FXIII_a) are activated by thrombin and Ca^{2+} in a manner similar to that for the plasma FXIII zymogen (1–10). Results described here indicate that human FXIII_a molecules derived from plasma, platelets, and placenta are apparently identical in primary structure. Although the terms FXIII_a² and FXIII_a₂ are used by various authors to refer to the activated plasma enzyme (2, 3), here we use FXIII_a to refer to the placental *a* chain subunit on which structural studies were done and no activity measurements made.

By methods of protein chemistry we have determined the complete amino acid sequence of human placental FXIII_a. Using our peptide sequences to design oligonucleotide probes, Grundmann *et al.* (11) have cloned the placental FXIII_a gene and determined its cDNA sequence. Prior to publication we exchanged data. Except for a few minor differences attributable to genetic polymorphism or to procedural problems, the amino acid sequence determined for the protein and that deduced from the cDNA are identical. On the basis of our studies and earlier data, we propose a molecular model for FXIII_a that shows the sites of activation and inactivation by thrombin and the location of the reactive thiol center and of a putative Ca^{2+} -binding domain.

MATERIALS AND METHODS

Materials. FXIII_a partially purified from pooled human placenta by Hermann Karges using the method of Bohn and Schwick (8), antisera specific for FXIII_a and for the *b* subunit of plasma FXIII, and human α thrombin were obtained from Behringwerke (Marburg, F.R.G.). The FXIII_a was purified further by HPLC. Plasma factor XIII was purified from Cohn fraction I of human plasma provided by Jerome Eckenrode (Michigan Department of Public Health, Lansing, MI) by a method modified from that of Cooke and Holbrook (12), in which a TSK G4000SW column (7.5 mm i.d. \times 60 cm, Anspec, Ann Arbor, MI) was used for separation of FXIII into the tetrameric form (a_2b_2) and the *b*₂ subunit. The latter was further purified on a TSK-gel Phenyl-5PW reversed-phase column (4.6 mm i.d. \times 7.5 cm) provided by Yoshio Kato of Toyo Soda (Shinnanyo, Yamaguchi, Japan).

Abbreviations: FXIII, zymogen of blood coagulation factor XIII; FXIII_a, activated FXIII (general); FXIII_a, the *a* subunit of FXIII; PIR-NBRF database, Protein Sequence Database from the Protein Identification Resource of the National Biomedical Research Foundation.

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Methods. Methods used for the determination of the primary structure of proteins in our laboratory have been described (13–16). For determination of free sulfhydryl groups, FXIIIa was modified with iodoacetic acid or 2-(bromoacetamido)-4-nitrophenol prior to reduction, and the modified FXIIIa was reduced and then aminoethylated. Limited digestion of FXIIIa was done with thrombin as described later. The Protein Sequence Database of the Protein Identification Resource, which was updated to April 1986, and the programs SEARCH, ALIGN, PRPLOT, CHOFAS, and DOTMATRIX were provided by the National Biomedical Research Foundation† (PIR-NBRF database).

RESULTS AND DISCUSSION

Amino Acid Composition, Polypeptide Structure, and Molecular Model. The *a* subunit of placental FXIII is an unglycosylated polypeptide chain consisting of 730 amino acid residues ($M_r = 83,005$) (Fig. 1). The amino end group of placental FXIIIa is blocked; presumably the amino-terminal serine is N-acetylated, as it is in human plasma and platelet FXIIIa (6) and in bovine plasma FXIIIa (7). The amino acid composition deduced from the protein sequence (Table 1) agrees well with that of Bohn and Schwick (8). It was surprising that no carbohydrate was detectable despite the presence of the six potential sites for N-glycosylation (see Table 1). Glucosamine was not present in any of the peptides isolated. Furthermore, in all peptides containing a potential N-glycosylation site, asparagine was clearly identified by automated sequence analysis, whereas a blank would have been obtained if the asparagine were glycosylated. We identified three free sulfhydryl groups in addition to the thiol at the active site. We found no direct evidence for disulfide bonds. This accords with reports that human plasma FXIIIa has no disulfide bonds (3, 17), but we have not yet established that all nine cysteines in placental FXIIIa have free thiol groups. Thrombin activation occurs by proteolytic cleavage at a single site after Arg-37, with release of the amino-terminal activation peptide. Thrombin inactivates FXIIIa by specific cleavage after Lys-541 to yield a 56-kDa fragment, which contains the reactive thiol site and a 24-kDa carboxyl-terminal fragment. These findings are summarized in the linear molecular model given in Fig. 1. The model also shows the location proposed for the Ca^{2+} -binding domain, the evidence for which is given later.

Amino Acid Sequence. The amino acid sequence of FXIIIa is given in Fig. 2 together with all evidence necessary to prove the structure. Sequence analysis was hindered by the blocked

Table 1. Amino acid composition of human placental FXIIIa based on the amino acid sequence determination

Amino acid	Residues, no.	Amino acid	Residues, no.
Aspartic acid	47	Valine	71
Asparagine	40	Methionine	18
Threonine	45	Isoleucine	38
Serine	46	Leucine	48
Glutamic acid	49	Tyrosine	29
Glutamine	26	Phenylalanine	32
Proline	33	Lysine	38
Glycine	50	Histidine	14
Alanine	37	Arginine	45
Cysteine	9	Tryptophan	15

The molecular mass of the unmodified polypeptide chain is 83,005; the number of residues is 730. Cys-314 has the active-site thiol; Cys-238, Cys-374, and Cys-695 have free sulfhydryl groups. Although no carbohydrate was detected in FXIIIa, there are six potential sites for N-glycosylation: Asn-17, Asn-46, Asn-541, Asn-556, Asn-613, and Asn-686.

end group and by the poor solubility of FXIIIa and the 56-kDa and 24-kDa fragments and their tendency to aggregate. Although the amino terminus of intact FXIIIa is blocked, an amino-terminal tryptic peptide with a free serine end group was obtained in a small amount but sufficient to prove the sequence. However, the last few residues of the FXIIIa sequence (from Arg-727 on) are uncertain because the carboxyl terminus of the protein appears to be heterogeneous. The amino acid sequence of FXIIIa is unique. Unlike many other plasma proteins (13–16, 19), FXIIIa does not exhibit significant internal homology in sequence that is detectable by the computer programs we used.

Comparison of the Protein Sequence with the Amino Acid Sequence Derived from cDNA. The sequence that we determined by amino acid sequence analysis of the FXIIIa protein is identical with that derived from the cDNA sequence (11) except for four positions, all of which are compatible with a single-base change. In addition, there is some heterogeneity at the carboxyl terminus of the protein. We have evidence for polymorphism at positions 77 and 78. There the sequence Arg-Arg is given by us in Fig. 2 and by Grundmann *et al.* (11), but we also found Gly-Lys in a chymotryptic peptide. At position 88 we found phenylalanine and not leucine as given by the cDNA sequence; leucine was not present in the tryptic and chymotryptic peptides that included this position. This difference may reflect genetic polymorphism or procedural problems. At position 651 we obtained glutamic acid rather than glutamine given by the cDNA sequence. Our result is based both on sequence analysis and on the specificity of V8

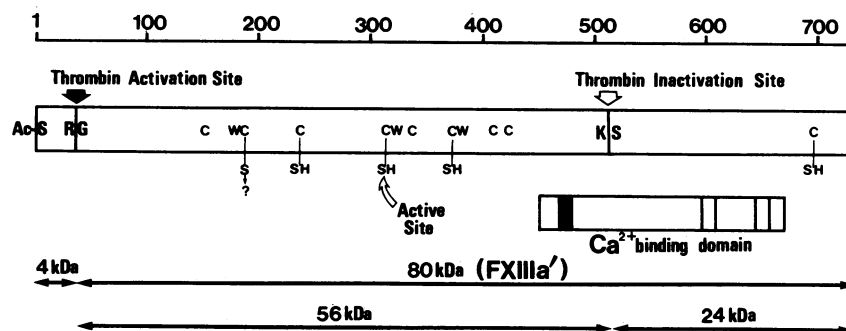


FIG. 1. Linear model of the primary structure of human placental FXIIIa showing the cleavage sites and the peptides formed by thrombin activation and inactivation. Amino acid residues discussed in the text are given in the one-letter code, and their position in the sequence of 730 residues is shown to scale. The four free sulfhydryl (SH) groups established by carboxymethylation are shown. The putative Ca^{2+} -binding domain contains three possible sites, the most probable of which is shaded (residues 468 to 479).

†National Biomedical Research Foundation (1986) Protein Sequence Data Base of the Protein Identification Resource (Washington, DC), Release No. 8.0.

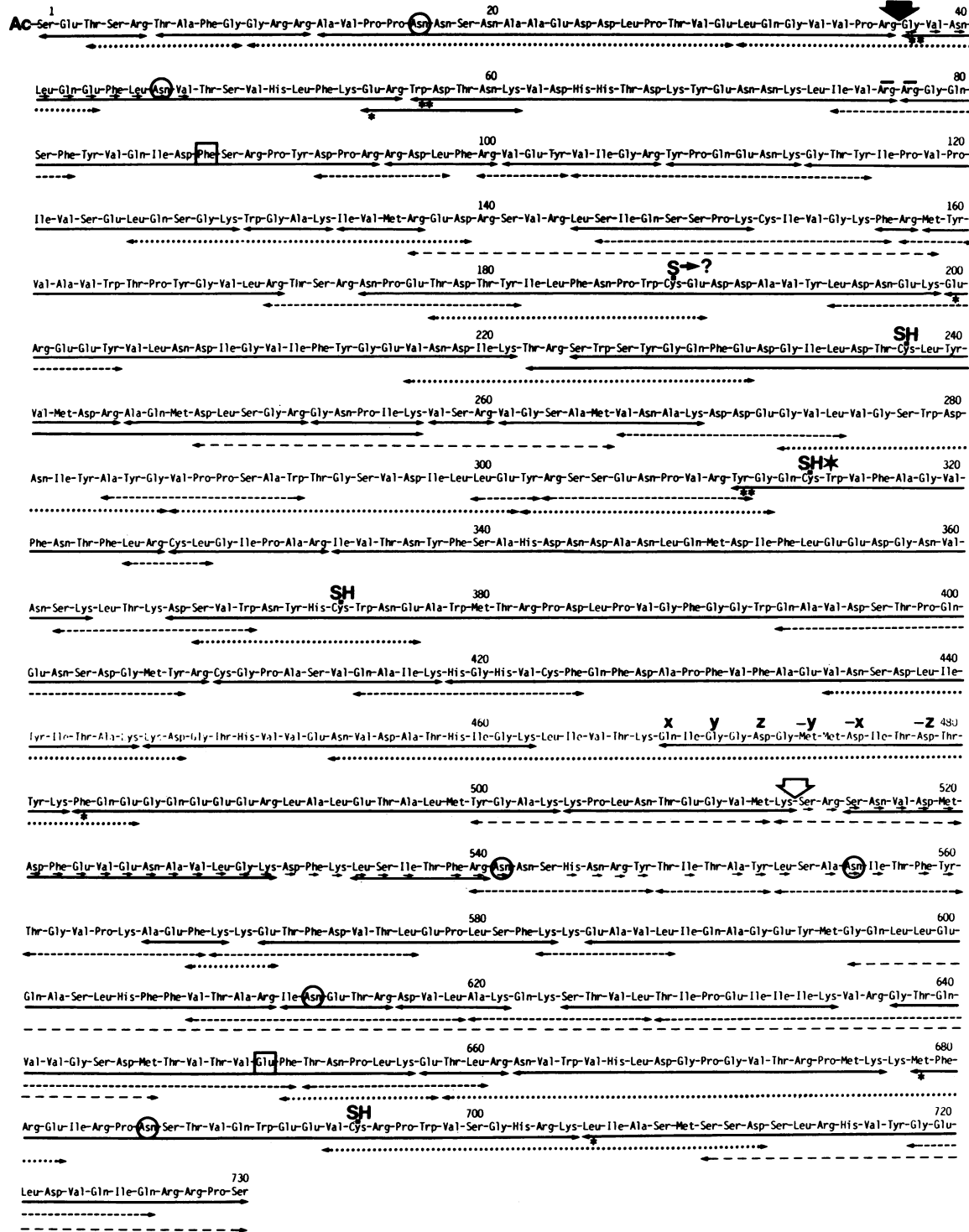


FIG. 2. Summary of the complete amino acid sequence of human placental FXIIIa. The sequence is shown with all peptides necessary for proof of sequence and is supported by all other peptides for which reliable data were obtained. The amino-terminal serine is blocked and probably is N-acetylated. The peptides were obtained by enzymatic digestion and CNBr cleavage (13–16) of the intact, reduced, and alkylated FXIIIa and of the 56-kDa and 24-kDa fragments. The peptides were purified by an automated HPLC system (18). The peptides obtained from different digestions are shown as follows: —, trypsin; *—, endoproteinase Lys-C; **—, endoproteinase Arg-C; ·····, *Staphylococcus aureus* V8 protease; - - - - - , chymotrypsin; - - - - - , CNBr. Potential glycosylation sites are circled, but no glucosamine was detected in any of the peptides. The thiol group at the active site (Cys-314) is indicated, and so are the free sulfhydryl groups identified at Cys-238, Cys-374, and Cys-695. Differences from the cDNA sequence are boxed. Possible ligands to Ca²⁺ are designated x, y, z, -y, -x, and -z.

protease. The difference can be explained either by deamidation or mutation of a single base. The carboxyl terminus of the FXIIIa protein was heterogeneous. Although methionine is deduced as the carboxyl-terminal residue from the cDNA sequence, the protein

sequence appeared to be heterogeneous at this end of the chain. Neither methionine nor homoserine was present in the carboxyl-terminal peptides; one chymotryptic peptide ended with Gln-726, other peptides ended with proline or serine, and hydrazinolysis suggested serine was the carboxyl termi-

nus of the protein. Such heterogeneity at the carboxyl terminus of proteins is being recognized increasingly and is attributable to posttranscriptional processing.

Proteolytic Cleavage of FXIIIa with Thrombin. Activation of FXIII requires cleavage by thrombin of the α subunit at a single site with release of the amino-terminal activation peptide. Our sequence for the activation peptide of placental enzyme agrees with that of Takagi and Doolittle (6) for the human plasma and platelet enzyme, except that we found 37 residues because of an additional valine in the sequence Val-Val-Pro-Arg at the carboxyl terminus. The activation cleavage site of the human enzyme from all three tissue sources is identical and occurs at an arginyglycine bond. The activated α subunit of plasma and platelet FXIII begins with the sequence Gly-Val-Asx-Leu-Glx-Glx (6). This corresponds to the sequence Gly-Val-Asn-Leu-Gln-Glu- that we found for thrombin-cleaved placental FXIIIa. Thus, FXIIIa enzymes from placenta, plasma, and platelets are identical in their amino-terminal sequence and site of action by thrombin.

A second specific cleavage by thrombin inactivates human plasma and platelet FXIIIa and produces a 56-kDa fragment that contains the reactive thiol group and also a 24-kDa fragment (10). We found that thrombin produces 56-kDa and 24-kDa fragments from human placental and plasma FXIIIa and that the cleavage pattern is similar for the enzyme from both sources (Fig. 3). The active thiol group is in Cys-314 in the placental 56-kDa fragment. Although no structural data are available for the plasma and platelet fragments, their known properties are similar to those obtained by thrombin cleavage of placental FXIIIa at the inactivation site.

The Active Site Peptide. The characteristic property used to identify transglutaminases is the Ca^{2+} -dependent covalent incorporation of amines via a cysteine active center that forms a thioester acyl intermediate (1-5). Both in human plasma FXIII (20) and in guinea pig liver transglutaminase (21), the tetrapeptide Gly-Gln-Cys-Trp has been identified as the site containing the active cysteine. This sequence is present at positions 312-315 in placental FXIIIa. When a 20-residue sequence from placental FXIIIa was used as a probe in a computer search of the PIR-NBRF database, the most homologous sequences were those around the active site of a series of thiol proteases (Fig. 4). These include the Ca^{2+} -dependent thiol proteases chicken calpain and rat cathepsins B and H as well as the plant thiol proteases papain

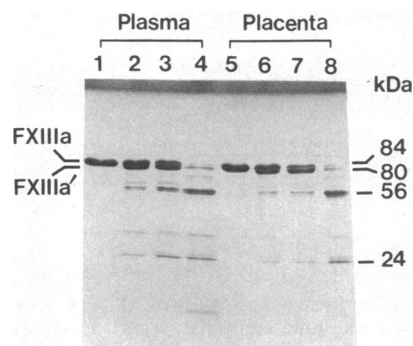


FIG. 3. Limited cleavage of human plasma and placental FXIIIa by thrombin. Conditions include an enzyme-to-substrate ratio of 5 units/1 mg in 50 mM Tris-HCl/0.1 M NaCl, pH 7.5 at room temperature, with the reaction stopped by addition of hirudin (Sigma). Incubation times were 0 min (lanes 1 and 5), 30 sec (lanes 2 and 6), 10 min (lanes 3 and 7), and 24 hr (lanes 4 and 8). For preparative purposes, the limited digest of placental FXIIIa was separated by TSK G4000SW column chromatography in the presence of 0.1% NaDodSO₄. The 56-kDa and 24-kDa fragments were concentrated and purified on a reversed-phase TSK-gel Phenyl-5PW reversed-phase column with a linear gradient of acetonitrile containing 0.1% trifluoroacetic acid.

Factor XIIIa	307-326	M	P	V	R	Y	G	Q	C	W	V	F	A	G	V	F	N	T	F	L	R
Calpain	101-120	C	Q	G	A	L	G	D	C	W	L	L	A	A	I	G	S	L	T	L	N
Cathepsin B	22-41	D	Q	G	S	C	G	S	C	W	A	F	G	A	V	E	A	M	S	D	R
CPase I	19-37	G	G	T	F	C	Q	A	C	W	R	F	A	G	T	C	G	P	Y	Y	*
Actinidin	18-37	S	Q	G	E	C	G	G	C	W	A	F	S	A	I	A	T	V	E	G	I
Papain	18-37	N	Q	G	S	C	G	S	C	W	A	F	S	A	V	V	T	I	E	G	I
Cathepsin H	19-38	N	Q	G	A	C	G	S	C	W	T	F	S	T	T	G	A	L	E	S	A

FIG. 4. Homology around the active thiol site of placental FXIIIa and of thiol proteases of various sources for which complete sequences are in the PIR-NBRF database. (CPase I is tomato carboxypeptidase inhibitor; its homology is probably not significant because the cysteine shown participates in a disulfide bond.)

and actinidin (and also ficin and bromelain, for which only the active site sequences are known). Using a consensus sequence for the active histidine site in thiol proteases, we could not identify a possible active histidine in FXIIIa. This fits with the fact that FXIIIa forms isopeptide bonds and does not cleave α -peptide bonds.

Other Cysteine Residues. By measurement of the incorporation of iodo[¹⁴C]acetic acid, Chung *et al.* (17) concluded that all of the free —SH groups of plasma FXIII are in the α chains and that each α chain has six —SH groups and no disulfide bonds. Others showed that there is a single reactive cysteine per α chain (3, 12, 20). Our sequence for placental FXIIIa contains nine cysteine residues. Four of these (Cys-238, Cys-314, Cys-374, and Cys-695) were carboxymethylated when the unreduced FXIIIa was treated with iodoacetic acid, and therefore they had free —SH groups. Cys-188 was not carboxymethylated in the unreduced protein; however, when the carboxymethylated protein was reduced and aminoethylated, Cys-188 formed aminoethylcysteine in high yield. This suggests Cys-188 was involved in an intra- or interchain disulfide bond. The status of the other cysteines has not yet been defined.

The Ca^{2+} -Binding Site(s). The active site —SH of thrombin-activated FXIIIa and of all other latent transglutaminases is unmasked only in the presence of Ca^{2+} (3, 5, 10, 12). Because the β subunits are not required for activation, the Ca^{2+} must bind to the α subunits and expose the active site by inducing conformational change. By a computer analysis of the amino acid sequence of FXIIIa, we have identified three potential Ca^{2+} -binding sites that are located in a putative Ca^{2+} -binding domain that overlaps the thrombin inactivation site (Fig. 1). The most probable binding site is in the sequence from Gln-468 to Asp-479 (Fig. 5). When the consensus sequence of the high-affinity Ca^{2+} -binding sites of calmodulin (22) was used to search for homology in the entire sequence of FXIIIa, the segment from Gln-468 to Asp-479 gave the highest score. Also, when 30-residue segments of FXIIIa were used to search the PIR-NBRF database for homology, many Ca^{2+} -binding proteins such as calmodulins and parvalbumins gave a high score for the sequence from Gln-468 to Asp-479. Two other segments of FXIIIa also showed homology, but they gave a lower score and are only illustrated schematically in Fig. 1.

The EF hand-type of high-affinity Ca^{2+} -binding site is characteristically shown as a loop flanked by two α -helices (22). According to secondary structure predictions (see below), the tight Ca^{2+} -binding site that we propose for FXIIIa (Gln-468 to Asp-479) is located in a series of β -turns that are flanked by a 10-residue β -sheet on the amino-terminal side and a 16-residue α -helix on the carboxyl side. This together with the fact that glycine replaces aspartic acid in the Y position explains the fact that the single Ca^{2+} -binding site of FXIIIa has a lower affinity constant (10^{-4} M) (23) than any of the four sites in calmodulins (10^{-6} M) (22). The binding of a single Ca^{2+} by FXIIIa is required for the unmasking of the

		X	Y	Z	-Y	-X	-Z
Factor XIIIa	468-479	Q	I	G	G	D	G
CaIM Human	129-140	D	I	D	G	D	G
Scallop	129-140	D	I	D	G	D	G
Tetrahymena	129-140	D	I	D	G	D	G
Dictyostelium	131-142	D	L	D	G	D	G
STNC Chicken	142-153	D	K	N	M	D	G
CaIM Scallop	56-67	D	A	D	G	D	G
Tetrahymena	56-67	D	A	D	G	D	G
CaBP beta Shrimp	113-124	D	V	D	G	D	G
alpha Shrimp	17-28	D	I	D	D	G	F
alpha Shrimp	113-124	D	V	N	G	D	G
PARV beta Pike	89-100	D	K	D	G	D	G

FIG. 5. Homology of the sequence Gln-468 through Asp-479 in FXIIIa to the sequences of high-affinity Ca²⁺-binding sites in calmodulins (CaIM), calcium-binding proteins (CaBP), skeletal troponin C (STNC), and parvalbumins (PARV) as revealed by a search of the PIR-NBRF database. The Ca²⁺ ion makes six ligand contacts with oxygen atoms in an octahedral vertex labeled X, Y, Z, -Y, -X, -Z, but some variation in the ligands is permissible (22).

active thiol at Cys-314 and, thus, must be accompanied by a major conformational change. The fact that the Ca²⁺-binding site is located close to the second site of thrombin cleavage may explain the inactivation incurred in this process.

Secondary Structure. No information is available from physicochemical measurements about the secondary structure of FXIIIa, so we used the computer programs PRPLOT and CHOFAS to predict the secondary structure by use of the Chou-Fasman parameters (24). Overall, the distribution predicted for the 730 residues is 36% β -pleated sheet, 35% β -turn, and 29% α -helix, or approximately one-third in each category. The activation peptide appears to have a random structure, and the Arg-Gly bond cleaved during thrombin activation is located in a β -turn. Up to the active site thiol (Cys-314), the polypeptide chain is predicted to have a structure composed predominantly of β -sheets separated by β -turns with only two or three strong nuclei for α -helices. The active-site Cys-314 begins a long hydrophobic β -sheet. The carboxyl-half of the chain seems to have a more organized structure than the first half. The thrombin inactivation site after Lys-513 is flanked by β -turns and is followed by a series of well-defined α -helices, β -sheets, and β -turns. The carboxyl terminus ends in a series of β -sheets and β -turns.

Properties of the b Chain of FXIII. In the course of purification of the a chain of human plasma FXIII, we also purified the b chain. We confirmed reports (3, 9, 10) that the b chain has an M_r of 85,000 in NaDodSO₄/PAGE and is immunologically unrelated to the a chain. The amino terminus is not blocked. The sequence of the first 34 residues is unrelated to that of the a chain and is Glu-Glu-Lys-Pro-X-Gly-Phe-Pro-His-Val-Glu-Asn-Gly-X-Ile-Ala-Gln-Tyr-X-Tyr-Thr-Phe-Lys-X-Phe-X-X-Pro-Met-Thr-Ile-Gly-Lys-Lys-

Identity of Placental, Plasma, and Platelet FXIIIa. All previous evidence indicates that human placental, plasma, and platelet FXIIIa are identical in physical, biological, and

immunochemical properties (3, 6, 8); our work suggests they are also probably identical in primary structure. We have now shown that placental FXIIIa is not distinguishable from the a chain of plasma FXIII in the following properties: (i) M_r in NaDodSO₄/PAGE, (ii) immunodiffusion, (iii) thrombin cleavage pattern and products, and (iv) amino acid sequence at the amino terminus and at the active site. Thus, it is likely that FXIIIa from all three tissue sources has the same primary structure.

Knowledge of the amino acid sequence of FXIIIa should greatly aid elucidation of its mechanism of enzymatic action and has facilitated cloning of the FXIIIa gene (11). Production of pure enzyme by recombinant DNA techniques would have direct application in replacement therapy for patients with congenital FXIIIa deficiencies (1, 9) and also would provide a source of FXIIIa for treatment of traumatic wounds and in postoperative surgery (9).

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- Lorand, L., Losowsky, M. S. & Miloszewski, K. J. M. (1980) in *Progress in Hemostasis and Thrombosis*, ed. Spaet, T. H. (Grune and Stratton, New York), pp. 245-290.
- Lorand, L., Credo, R. B. & Janus, T. J. (1981) *Methods Enzymol.* **80**, 333-341.
- Folk, J. E. & Finlayson, J. S. (1977) *Adv. Protein Chem.* **31**, 1-133.
- Folk, J. E. (1980) *Annu. Rev. Biochem.* **49**, 517-531.
- Lorand, L. & Conrad, S. M. (1984) *Mol. Cell. Biochem.* **58**, 9-35.
- Takagi, T. & Doolittle, R. F. (1974) *Biochemistry* **13**, 750-756.
- Nakamura, S., Iwanaga, S. & Suzuki, T. (1974) *Biochem. Biophys. Res. Commun.* **58**, 250-256.
- Bohn, H. & Schwick, H. G. (1971) *Arzneim-Forsch.* **21**, 1432-1439.
- Bohn, H. (1972) *Ann. N.Y. Acad. Sci.* **202**, 256-272.
- Schwartz, M. L., Pizzo, S. V., Hill, R. L. & McKee, P. A. (1973) *J. Biol. Chem.* **248**, 1395-1407.
- Grundmann, U., Amann, E., Zettlmeissl, G. & Küpper, H. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8024-8028.
- Cooke, R. D. & Holbrook, J. J. (1974) *Biochem. J.* **141**, 79-84.
- Lozier, J., Takahashi, N. & Putnam, F. W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3640-3644.
- Takahashi, N., Ortel, T. L. & Putnam, F. W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 390-394.
- Takahashi, N., Takahashi, Y. & Putnam, F. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 73-77.
- Takahashi, N., Takahashi, Y. & Putnam, F. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1906-1910.
- Chung, S. I., Lewis, M. S. & Folk, J. E. (1974) *J. Biol. Chem.* **249**, 940-950.
- Takahashi, N., Ishioka, N., Takahashi, Y. & Putnam, F. W. (1985) *J. Chromatogr.* **316**, 407-418.
- Doolittle, R. F. (1984) in *The Plasma Proteins*, ed. Putnam, F. W. (Academic, Orlando, FL), 2nd Ed., pp. 317-359.
- Holbrook, J. J., Cooke, R. D. & Kingston, I. B. (1973) *Biochem. J.* **135**, 901-903.
- Folk, J. E. & Cole, P. W. (1966) *J. Biol. Chem.* **241**, 3238-3240.
- Klee, C. B. & Vanaman, T. C. (1982) *Adv. Protein Chem.* **35**, 213-321.
- Lewis, B. A., Freyssinet, J.-M. & Holbrook, J. J. (1978) *Biochem. J.* **169**, 397-402.
- Chou, P. Y. & Fasman, G. O. (1978) *Annu. Rev. Biochem.* **47**, 251-276.