Three-dimensional structure of a transglutaminase: Human blood coagulation factor XIII

(x-ray crystallography/plasma transglutaminase/fibrinolyticase/fibrin-stabilizing factor)

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ABSTRACT Mechanical stability in many biological materials is provided by the crosslinking of large structural proteins with γ -glutamyl- ϵ -lysyl amide bonds. The three-dimensional structure of human recombinant factor XIII (EC 2.3.2.13 zymogen; protein-glutamine:amine γ -glutamyltransferase a chain), a transglutaminase zymogen, has been solved at 2.8-Å resolution by x-ray crystallography. This structure shows that each chain of the homodimeric protein is folded into four sequential domains. A catalytic triad reminiscent of that observed in cysteine proteases has been identified in the core domain. The amino-terminal activation peptide of each subunit crosses the dimer interface and partially occludes the opening of the catalytic cavity in the second subunit, preventing substrate binding to the zymogen. A proposal for the mechanism of activation by thrombin and calcium is made that details the structural events leading to active factor XIIIa'.

Transglutaminases (protein-glutamine:amine γ -glutamyltransferases, EC 2.3.2.13) are thiol enzymes that catalyze the formation of amide crosslinks between glutamine and lysine residues in a variety of proteins, usually to provide structural stability in biological materials (1). Members of this class have been isolated from a variety of tissue and cell types such as plasma, platelets, keratinocytes, and endothelial cells. The reaction catalyzed is shown in Eq. 1:

We present here the three-dimensional model of a member of the transglutaminase family, that of human blood coagulation factor XIII.

Factor XIII is the last enzyme to be activated in the blood coagulation cascade. The active form, factor XIIIa', catalyzes crosslinking between fibrin molecules, which increases the mechanical strength of blood clots and renders them less susceptible to proteolytic cleavage (2–4). This enzyme also crosslinks fibrin to the adhesive glycoproteins fibronectin and thrombospondin to anchor the blood clot to the site of injury (5, 6), fibrin to α_2 -antiplasmin to increase the resistance of the clot to plasmin (7), and fibrin to other coagulation

substrates such as factor V, platelet actin, and von Willebrand factor (8, 9). Individuals who are deficient in factor XIII characteristically rebleed after initial clot formation; other typical symptoms are umbilical and intracranial bleeding in children, a 100% spontaneous abortion rate in women, and sterility in men (10). As well as being a critical component of the blood coagulation system, factor XIIIa' also crosslinks fibronectin, vitronectin, collagen, and lipoprotein(a) in the extracellular matrix (11–14) and is important in the stimulation of connective tissue cells and the steering of their processes involved in inflammation and wound healing. Abnormal levels of factor XIII or of factor XIIIa' activity have been linked to a variety of diseases, among them ulcerative colitis and other gastrointestinal disorders (15, 16), liver cirrhosis and acute leukemia (17), rheumatoid arthritis (18), retinal disease (19), sclerosis (20), atherosclerosis, and heart disease (21–23). Often symptoms of these diseases can be alleviated by treatment with factor XIII concentrate. In combination with fibrinogen, thrombin, and other clotting proteins, factor XIII is a component of "fibrin glue," a tissue adhesive that is used as a replacement or adjunct to conventional sutures (24). Finally, macrophages associated with some cancerous tumors have been found to contain factor XIII and have been shown to be involved in the deposition of crosslinked fibrin or fibrinogen in breast cancer and Hodgkin disease (25, 26).

In platelets, placenta, and other tissues, factor XIII is a dimer of two a chains, each containing 730 amino acid residues (27), while in the circulating plasma it is a heterotetramer of two a and two b subunits. Both forms of factor XIII are activated by thrombin in the presence of Ca^{2+} with release of a 37-residue amino-terminal peptide; in the case of the plasma form, dissociation of the b subunits occurs as well. We describe in this work the results of our x-ray diffraction studies of recombinant human factor XIII_a and the structural model determined at 2.8-Å resolution.[§]

MATERIALS AND METHODS

Recombinant human factor XIII was obtained from *Saccharomyces cerevisiae* (28) in its zymogen form, which is a dimer containing two a subunits with a total molecular weight of 166 kDa. Crystals of the protein grown from ammonium sulfate at pH 5.8 are orthorhombic (space group $P2_12_12_1$; cell dimensions $a = 101.2$ Å, $b = 182.7$ Å, and $c = 93.4$ Å) (29). There are two monomers in the asymmetric unit, giving a calculated value of 53% for the solvent content.

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[§]The atomic coordinates have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 (reference 1 GGT). This information is embargoed for 1 year from the date of publication.

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Using multiple isomorphous replacement (MIR) techniques, starting phases obtained from five heavy atom derivatives were used to calculate 2.9-Å resolution electron density maps (Tables 1 and 2). Phases derived from an initial polyalanine model were combined with MIR phases to yield an improved 2.8-Å resolution map from which the final model was built. An *R* factor of 0.434 (10.0- to 2.8-Å resolution) for native data collected at a synchrotron source was calculated for this initial model. Preliminary refinement by conjugate gradient minimization has been carried out with the program X-PLOR (35). At the current stage of refinement [*R* = 0.214 for data from 10.0- to 2.8-Å resolution, *F* > 2σ(*F*), with 41,504 reflections and 11,366 atoms], the model has good geometry [rms deviation from ideality = 0.018 Å (bonds) and 3.9° (angles)], and the average value of the individually refined *B* factors is 24.4 Å².

RESULTS

Subunit Structure. Our current x-ray model of the factor XIII a subunit consists of residues 8–727 with one gap between residues 30 and 43. The two crystallographically independent subunits are very similar: the rms difference between their α-carbon atoms is 0.61 Å for the large fragment of the model (which contains residues 43–727) and 1.32 Å for the shorter 23-residue amino-terminal fragment. As shown in Fig. 1, four distinct and sequential domains make up the factor XIII a monomer: the β-sandwich, core, barrel 1, and barrel 2. In three of these domains, the secondary structure is predominantly β-sheet, while the remaining domain is of mixed α-helix and β-sheet. Thus, the subunit can be thought of as a flattened triangular wedge about 100 Å long, 60 Å wide, and 50 Å in the third dimension, with a β-sheet domain placed at each vertex and the mixed helix and β-sheet domain at the core.

Activation Peptide. Situated at the amino terminus is a short activation peptide. Activation by thrombin cleavage occurs at Arg-37, which is in the gap of 12 residues that could not be located in the various electron-density maps. The 23-residue section of the activation peptide in the model does not possess any regular secondary structure. Half a dozen short oxygen-to-nitrogen hydrogen bonds, with distances less than or equal to 2.9 Å, hold residues Asn-17 through Thr-28 of the activation peptide to the first domain of the a subunit, the β-sandwich.

Domain Structure. The β-sandwich domain consists of 140 residues from Glu-43 through Phe-184 and is characterized by two four-stranded antiparallel sheets twisted about 50° with respect to each other. There are only two turns of helix in this domain; they make up part of a 28-residue loop between the

Table 2. Heavy-atom phasing

Derivative	Sites, no.	Useful resolution limit, Å	<i>R</i> (centric)*	Average phasing power
Uranyl crystal 1				
Isomorphous	15	2.9	0.581	2.35
Anomalous	15	2.9	—	1.64
Uranyl crystal 2				
Isomorphous	13	3.6	0.587	1.84
Anomalous	13	5.0	—	1.24
PIP	12	4.6	0.524	1.70
K ₂ PtCl ₄	12	3.1	0.621	1.47
EuCl ₃	3	4.5	0.628	1.46
YbCl ₃	4	3.2	0.628	1.70

Refinement of heavy-atom parameters was carried out by using PHASES (31). The combined figure of merit before solvent flattening was 0.67; that after solvent flattening was 0.79 (32). Only data with *F* > 2σ(*F*) were used. Density averaging techniques exploiting the noncrystallographic 2-fold symmetry were also employed; map calculations were carried out with the CCP4 package of crystallographic software (33). The program o was used for model building (34). Isomorphous and anomalous refer to isomorphous and anomalous differences between native and heavy-atom derivative data; unless indicated, phasing information is based on isomorphous differences.

**R*(centric) = Σ_h(|*F*_{PH} − *F*_P| − |*f*_H|)/Σ_h|*F*_{PH} − *F*_P|. Phasing power = *f*_{rms}/*E*_{rms}, where *f*_{rms} = [(Σ_h*f*_H²)/*n*]^{1/2} and *E*_{rms} = [Σ(*F*_{PH} − |*F*_P + *f*_H)²/*n*]^{1/2}.

first and second β-strands of the sandwich. Overall, this domain is about 40 Å in each dimension. Two other domains that are of predominantly β structure have a seven-stranded β-barrel fold and are located at the carboxyl terminus of the monomer. There are about 100 residues in each of these domains: barrel 1 contains residues Ser-516 through Thr-628 and is a little larger than barrel 2, which runs from Ile-629 through Arg-727. Each barrel is just under 50 Å long and about 25 Å wide. These domains have the “3+4” sheet structure that distinguishes the fibronectin III fold from the “4+3” β-sheet pattern seen in the immunoglobulin constant domains (37). The two barrels in the factor XIII a subunit are quite similar (the rms difference between 81 equivalent α-carbon atoms within 3.0 Å is 1.57 Å); the larger number of residues in barrel 1 is due to two connecting segments that loop away from the body of the barrel more than any observed in barrel 2. One of these loops is between strands 3 and 4 and contains a tyrosine at position 560 whose significance will be discussed later. While barrel 1 and barrel 2 are structurally very similar, there is no significant se-

Table 1. Data collection

Data set	Resolution limit, Å	Measurements, no.	Unique reflections	% complete	<i>R</i> (merge)*
Native					
Siemens, 6 crystals	2.4	490,649	44,344	63.3	0.123
SSRL	2.4	217,433	65,937	94.1	0.086
Derivative					
Uranyl nitrate					
Crystal 1	2.9	145,075	62,858	85.6	0.095
Crystal 2	3.4	65,429	34,868	85.0	0.077
PIP	3.4	63,245	32,578	76.3	0.086
K ₂ PtCl ₄	3.1	86,405	45,383	76.1	0.094
EuCl ₃	2.8	39,950	36,410	47.1	0.081
YbCl ₃	3.0	49,872	41,309	74.3	0.053

Early native data and all heavy-atom derivative data used in phasing were collected on a Siemens area detector equipped with a Rigaku RU200 rotating-anode generator and a graphite monochromator. Data processing was carried out by using the XENGEN package (30) and the program MACRO (S. T. Rao, J. P. McAlister, and E. A. Merritt, personal communications). A native data set was also collected on Beamline VII-1 at the Stanford Synchrotron Radiation Laboratory (SSRL).

**R*(merge) = Σ_hΣ_i|*I*_{h,i} − ⟨*I*_h⟩|/Σ_hΣ_i|*I*_{h,i}|, where *h* measurements are unique reflection indices (*I*) and *i* represents symmetry equivalent indices. PIP, di-μ-iodo-bis(ethylenediamine)diplatinum(II) nitrate.

FIG. 1. Stereo diagram showing the overall and secondary structure in the factor XIII a monomer (36). From the amino terminus, the four domains are the β -sandwich (cyan), the core domain (pink), barrel 1 (green), and barrel 2 (yellow). The one gap in the model occurs between the amino terminus activation peptide (dark blue) and the β -sandwich.

sequence similarity between the two domains, and neither is similar in sequence to fibronectin type III domains (38).

The largest and central domain in the factor XIII a subunit spans about 330 amino acid residues, from Asn-185 near the β -sandwich domain to Arg-515, after which barrel 1 begins. In this core domain, there are 10 short segments of α -helix; a two-turn helix is found in a loop near the β -sandwich, while the other helices are arranged in two groups, which are separated by a central β -sheet. This domain thus has several distinct structural features. At its amino-terminal end, close to the β -sandwich, are five helices. In this region two antiparallel β -strands that flank barrel 2 approach the second feature of the core, a twisted β -sheet consisting of six antiparallel strands located at the center of the domain. In turn, the β -sheet is curled along the side of the third region in the core, which contains the remaining four helical segments. The other prominent secondary structure in this region is a three-stranded mixed β -sheet near the carboxyl terminus of the core that flanks one end of barrel 1. It is in this domain that we have located the active site of the enzyme, which is described in a separate section.

Conserved Residues. Amino acid sequence alignments of 15 members of the transglutaminase family, including the non-enzymatic human erythrocyte membrane protein band 4.2, reveal that the proteins share 25–45% identity with factor XIII. The distribution of conserved residues in members of this family among the four structural domains has been examined by sequence alignments.[†] A small number of the conserved residues are located in the β domains, but a dramatic majority (136 of 180) are found in the central-core domain. This result indicates that the fold of the core domain and the catalytic mechanism remain consistent throughout the transglutaminase family and implies that the differing substrate specificity among the transglutaminases is localized in the bordering domains.

Dimer Structure. Each of the two factor XIII subunits comes together with a crystallographically related mate to form what we believe is the physiological dimer. Two orthogonal views of the dimer, represented as peptide coils, are given in Fig. 2. The dimer is formed when the long edges of two subunits align

and thus has a hexagonal shape in one view (Fig. 2 *Left*). There is a long interface between the monomers that involves both sandwich and core domains. Accessible surface area calculations (39) give values of 1710 and 1880 Å² for the area buried upon formation of the two crystallographically independent dimers. These numbers are comparable to those reported for other protein–protein complexes (40). An interesting feature of the dimer is that the activation peptide of each subunit crosses the interface to associate with the core in the other half of the dimer; in so doing, the peptide passes over the entrance to the catalytic cavity in the second monomer and precludes substrate binding. Five short hydrogen bonds hold each activation peptide to the other subunit in the dimer. A second view (Fig. 2 *Right*) of the dimer shows that the hexagon is not flat but puckered. Both activation peptides cross the large (90 Å wide) concave top face; it is from this face that the active site cavities of each subunit are accessible.

Active Site. A small number of residues that are conserved in the active transglutaminases come together to form the active site (Fig. 3). At the amino terminus of the longest helix in the structure sits Cys-314, which is known to be essential for the enzyme's catalytic activity (41). This helix is bordered on one side by the central twisted β -sheet in the core domain. The sulfhydryl group of Cys-314 forms a hydrogen bond to His-373 (the S^γ–N^{δ1} distances in the two crystallographically independent monomers are 3.2 and 3.4 Å), which is in a strand in the middle of the β -sheet. In turn, the other nitrogen atom in the histidine ring forms a hydrogen bond with a terminal oxygen atom of Asp-396 (N^{ε2}–O^δ = 2.9 and 3.0 Å), a residue that sits on a neighboring β -strand in the same sheet. Side-chain atoms of this Cys-His-Asp triad can be easily superimposed onto those for the Cys-His-Asn triad in papain (rms fit of 0.61 and 0.49 Å). Thus, the catalytic triad we observe in this transglutaminase is very similar to the arrangement in papain's cysteine protease family (unpublished data).

In factor XIII, these three catalytic residues are at the base of a cavity bounded by the core and barrel 1 domains. Also participating in the hydrogen-bonding pattern in the active site cavity is a tyrosine residue in one of the long loops in the barrel 1 domain: the distance from the hydroxyl oxygen atom of Tyr-560 to the sulfur atom of Cys-314 is 2.9 and 3.1 Å for the two subunits. Such OH...S hydrogen bonds are unusual and generally found only if the sulfur atom is negatively charged (42, 43). This result suggests that the hydrogen-bonding interaction between Cys-314 and His-373 is that of a thiolate–imidazolium ion pair as in the cysteine proteases (44). Also in Fig. 3 are a group of conserved residues surrounding His-342, which forms a hydrogen bond to Glu-

[†]Amino acid sequences for the following members of the transglutaminase family were used: factor XIII, human and mouse tissue transglutaminase, guinea pig tissue transglutaminase, bovine endothelial transglutaminase, chicken erythrocyte transglutaminase, human and rat keratinocyte transglutaminase, human and rat prostate transglutaminase, human and mouse epidermal transglutaminase (transglutaminase 3), *Limulus* hemocyte transglutaminase, anulin, and human p4.2 protein (unpublished data).

FIG. 2. Two orthogonal views of the factor XIII dimer, represented as coil structures. The activation peptide and domains in one subunit are colored as in Fig. 1, with the addition that the peptide linking the core and barrel 1 domains is in dark blue. For the second subunit in the dimer, the four domains are in light grey; both the activation peptide and the peptide linking the core domain to barrel 1 are in dark grey. The location of the active site in each subunit is marked by a red asterisk.

434 ($N^{\delta 1}-O^{\epsilon} = 2.6$ and 2.7 Å). The $N^{\epsilon 2}$ atom of His-342 has no hydrogen bond to residues in the immediate area, and its distance to Cys-314 ($N^{\epsilon 2}-S^{\gamma} = 8.1$ and 8.3 Å) is too far for participation in a second catalytic triad. Asp-343 is hydrogen-bonded to Arg-11' in the activation peptide of the second subunit in the dimer. In the following discussion, we hypothesize that the pair of residues, His-342 and Asp-343, guide the lysyl substrate residue into the active site.

DISCUSSION

The transamidation reaction catalyzed by factor XIII can be thought of as being similar to the reverse of proteolytic cleavage but differing in that water must be excluded from the reaction to avoid deamidation side reactions. Our x-ray studies identify a catalytic triad that resembles that of the cysteine proteases, and the arrangement of the helix and four chains of β -sheet bearing the catalytic triad residues indicates that factor XIII shares a common ancestor with the cysteine proteases (unpublished data). The active site is less accessible than that of the proteases, consistent with observations that many inactivators of the cysteine proteases do not react with transglutaminases (45). Another intriguing feature of this transamidation is that it involves two very large protein molecules as substrates. After removal of the activation peptide, the active-site cavity can be accessed from two directions. We postulate that the catalytic Cys-314 can be approached by the glutamine substrate macromolecule from the direction of the two β -barrel domains (from the left in Figs. 3 and 4), most likely displacing Tyr-560 by movement of barrel 1, and possibly barrel 2 as well. A second large substrate (the lysyl substrate) can access the active-site cavity from the opposite direction, either from a long groove

that runs along the subunit-subunit interface or by bending along the β -sandwich domain.

Our structural studies suggest a sequence of events, among them movement of one or both of the barrel domains, which likely occur upon activation of the zymogen in the presence of calcium. In the factor XIII zymogen, the catalytic triad residues are not accessible to substrate (Fig. 4). The first step in the activation of factor XIII, then, requires the movement of the activation peptide from the active site cavity after thrombin proteolysis at Arg-37.

Three pieces of information are consistent with additional structural rearrangements being connected with enzymatic activity. One is that transglutaminase 3 is activated 15-fold by proteolysis of the linker peptide joining the core and barrel 1 domains (46). In this enzyme the linker is longer than in the other transglutaminases and indicates that the length of this segment is critical to enzyme activity. Second is the observation that a 52-kDa thrombin or trypsin-cleaved factor XIII fragment (corresponding to the core and sandwich domains) possesses catalytic activity (47). This demonstrates that the core domain retains its structure independently of the barrel domains. Finally, prolonged incubation of factor XIII with thrombin leads to a Ca^{2+} -dependent cleavage at Lys-513 (47, 48). In the zymogen structure, this residue is located in the peptide linking the core domain to barrel 1. The residue points into the protein, participating in a salt bridge with Glu-453, while its nonpolar neighbors Val-511 and Met-512 are directed out toward solvent. We speculate that upon binding of Ca^{2+} and substrate, the peptide linking the core and barrel 1 domains undergoes a conformational change. This movement causes the barrel 1 domain or possibly both

FIG. 3. Stereoview of the active-site cavity of one α subunit in the factor XIII dimer. The backbone of the protein is shown as a coil structure, with secondary structure features drawn as thick helices and arrows. Domains are colored as in Fig. 2 (core domain in pink, barrel 1 in green, and the activation peptide of the second subunit in the dimer as dark grey). Side-chain atoms of catalytic triad residues (Cys-314, His-373, and Asp-396), several other active site residues taking part in interesting hydrogen-bond interactions (Tyr-560, His-342, Asp-343, and Glu-434), and Arg-11' in the activation peptide of the second subunit in the dimer are drawn as ball-and-stick structures (grey = carbon, blue = nitrogen, red = oxygen, and yellow = sulfur).

FIG. 4. Stereoview of the active site of one subunit in the factor XIII dimer. Side-chain atoms of the catalytic triad residues (Cys-314, His-373, and Asp-396), Tyr-560 from barrel 1, and Arg-11' from the activation peptide of the second subunit in the dimer are shown. The catalytic triad residues become accessible to substrate upon removal of (i) the second subunit's activation peptide (dark grey) from the opening to the active-site cavity and (ii) Tyr-560 from the nucleophilic Cys-314 of the triad. Removal of the activation peptide occurs upon cleavage by thrombin at a site in the gap between the activation peptide and the rest of the second subunit (light grey). Residue Tyr-560 is removed from Cys-314 by shifting barrel 1 (green) away from the active site. Movement of barrel 1, with or without barrel 2 (yellow) as well, may occur upon a conformational change of the peptide (dark blue) linking the core domain (pink) to barrel 1.

β -barrels to rotate away from the core, making Lys-513 accessible for proteolysis by thrombin.

The movement of the barrel domains removes Tyr-560 from the glutamine binding pocket, allowing the glutamyl substrate access to Cys-314 so that the acyl enzyme can be formed and NH_3 can diffuse away from the enzyme. The lysyl substrate then approaches along either the β -sandwich domain or the long subunit interface groove, where it is steered to the active site by Asp-343 and the His-342/Glu-434 diad. Finally, reaction of the lysyl substrate occurs, and the crosslinked product then diffuses away.

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