Structure and function of factor XI

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Introduction

Factor XI (FXI) is the zymogen of a blood coagulation protease, factor XIa (FXIa), that contributes to hemostasis through activation of factor IX (FIX; Figure 1).1-3 The protein is a 160-kDa disulfide-linked dimer of identical 607 amino acid subunits, each containing 4 90- or 91-amino acid repeats called apple domains (A1 to A4 from the N-terminus) and a C-terminal trypsin-like catalytic domain.3-7 The structure is distinctly different from those of the well-characterized vitamin K–dependent coagulation proteases.1 FXI circulates in blood as a complex with high molecular weight kininogen (HK),8 prekallikrein (PK), the zymogen of the protease α-kallikrein, is a monomeric homolog of FXI with the same domain structure9,10 that also circulates in complex with HK.11 A recent analysis of vertebrate genomes confirmed that FXI and PK are products of a duplication event during mammalian evolution.12 The ancestral predecessor is also a protease with 4 apple domains, but its functional properties have not been studied.12

In the cascade/waterfall models of coagulation that are the basis for the activated partial thromboplastin time (aPTT) assay, FXI activation by FXIa initiates fibrin formation (Figure 1).1 However, newer schemes do not assign FXI a role in early fibrin generation, based largely on the observation that FXI deficiency causes relatively mild bleeding.1-3 FXIa is now postulated to be part of a feedback loop that sustains thrombin generation through FIX activation to consolidate coagulation (Figure 1).13-15 This appears to be particularly important in tissues with robust fibrinolytic activity, such as the oropharynx and urinary tract, which are common sites of bleeding in FXI-deficient patients.2,16-23

Congenital FXI deficiency is associated with a variable, mild to moderate bleeding disorder.2,16,17 Severe deficiency (< 15% of normal plasma activity) is prevalent in people of Jewish ancestry.2,16,17,24 The carrier rate is approximately 5% in Ashkenazi Jews, with severe (homozygous) deficiency found in 1 in 450 persons.16,24 Two mutations account for more than 90% of abnormal alleles in this population.24,25 The severe mutation Glu117Stop encodes a truncated protein,25 and homozygotes lack plasma FXI antigen. The more subtle missense mutation Phe283Leu causes a defect in FXI dimer formation.26-28 More than 180 other FXI gene mutations associated with FXI deficiency have been reported (http://www.factorxi.org, http://www.isth.org),29 including more than 100 single amino acid (missense) substitutions. Congenital deficiencies of vitamin K–dependent coagulation proteases are often associated with dysfunctional protein in plasma (cross-reactive material positive [CRM+] deficiency).30 In contrast, most cases of FXI deficiency are associated with low plasma levels of FXI protein (CRM− deficiency).30

There has been renewed interest in FXI as a result of population-based studies and work with animal models strongly indicating that this protein makes important contributions to arterial32-36 and venous37 thrombosis, ischemia-reperfusion injury in the central nervous system,38 and the pathology of sepsis.39 The observation that deficiency or inhibition of FXI interferes with platelet accumulation in growing thrombi33,35 has led to a reanalysis of the mechanisms involved in FXI activation and its interactions with platelets. In this review, we present a summary of recent structural data on FXI as it relates to current knowledge of protease function and to congenital FXI deficiency.

FXI structure

Crystal structures are available for full-length zymogen FXI,7 and the isolated active protease domain of FXIa in complex with
natural and synthetic inhibitors. The apple domains of FXI and PK are members of the PAN (plasminogen, apple, nematode) module family, with homology to the N-terminal domains of hepatocyte growth factor and plasminogen. Each apple domain consists of a 7-stranded β-sheet that folds into a curved antiparallel sheet cradling an α-helix (Figure 2A). Two disulfide bonds lock the helix onto the central β4 and β5 strands, whereas a third connects the N- and C-termini of the domain. This core topology is equivalent to PAN domains from hepatocyte growth factor, leech antiplatelet protein, and microneme antigen EtMIC5. The β4-β5 loop and β5-β6 crossover loop generate a small pocket on the opposite side of the sheet from the α-helix (Figure 2A).

A remarkable feature of the FXI structure, which it probably shares with PK, is the intimate linkage of the apple domains into a disk-like platform around the base of the catalytic domain (Figure 2B). The disk has 2 sets of topologically equivalent interfaces. The side interfaces between A1 and A2 and between A3 and A4 are substantial, burying surface areas of 441 Å² and 444 Å², respectively. In comparison, the end interfaces between A1 and A4 (380 Å²) and between A2 and A3 (284 Å²) are smaller. The FXI subunit, therefore, is compact compared with the vitamin K–dependent proteases, whose elongated structures serve to position the catalytic domains above phospholipid membranes. Amino acid substitutions in the apple domains of FXI-deficient patients are shown in Figure 2C. With few exceptions, these mutations involve residues that are conserved in FXI, PK, and their ancestral predecessor (supplemental Table 1, available on the Blood website; see the Supplemental Materials link at the top of the online article). Many involve buried residues (green in Figure 2C) that probably affect apple domain folding or disrupt formation of interdomain interfaces, which would explain why the majority of cases of FXI deficiency are CRM⁻.
FXI deficiency interfere, or are predicted to interfere, with dimerization. The best characterized are Phe283Leu,26-28 which causes a partial dimerization defect, and Gly350Glu,27 which prevents dimer formation. Riley et al showed that the Phe283Leu substitution causes increased dimer dissociation and stabilizes the monomer through altered side-chain packing that is unfavorable for dimer formation.28

The dimeric structure of FXI, unique among coagulation proteases, has implications for inheritance of FXI deficiency. Although Glu117Stop and Phe283Leu are associated with a bleeding diathesis inherited in a dominant manner,1 abnormal subunit. The mutant subunit, in effect, traps wild-type subunits by forming nonsecretable heterodimers with 1 normal and 1 abnormal subunit. The mutant subunit, in effect, traps wild-type subunits within the cell. This mechanism has been demonstrated in deficiencies of multimeric proteins, such as von Willebrand factor6 and fibrinogen.53 The FXI missense mutations Ser225Phe, Cys398Tyr, Gly400Val, and Trp569Ser exhibit a dominant negative effect when coexpressed with wild-type FXI and are associated with FXI deficiency that may be inherited in a dominant manner.27,54 It is probable that these mutations do not perturb apple domain disk structure significantly, allowing dimerization to occur. In this regard, it is interesting to note that 3 of 4 mutations known to operate by a dominant negative mechanism involve residues in the catalytic domain.

The structure of the FXI dimer is shown in Figure 3 with highlights on residues implicated in zymogen activation, substrate recognition, and receptor binding (also shown in Figure 2B). Many of the complex protein-protein interactions involving FXI and FXIa are mediated by its apple domains. Binding sites for HK,10,55,56 thrombin,7,57 heparin,58-61 FIX,62,63 and platelet receptors, such as glycoprotein Ib (GPIb),64 will be discussed in the following sections.

FXI and PK binding to HK

HK is a 120-kDa multifunctional plasma protein with 6 domains, designated D1 to D6.65 Almost all FXI1 and 75% to 90% of PK11,66 circulate in a complex with HK. The D6 domain mediates binding to both proteins.65 The physiologic importance of the PK-HK interaction seems clear as cleavage of HK by α-kallikrein (activated PK) liberates the vasoactive peptide bradykinin.65 The importance of the FXI-HK interaction is not as evident, as HK deficiency is not associated with a bleeding disorder.66 In the presence of zinc ions, HK is required for optimal FXI binding to GPIb on activated platelets in suspension, enhancing the binding stoichiometry by approximately 2-fold.67,69 However, FXI binding to platelets assayed under flow (discussed in “FXI interactions with platelet receptors”) is not enhanced by HK.70

The sites on FXI and PK that bind HK are probably similar. Studies using FXI/PK chimeras, individual apple domains, and short peptide sequences derived from apple domains indicate the A2 domain is required for HK binding, with additional contributions from A1 and A4.55,56,71 Detailed mutagenesis to localize the binding site has not been performed; however, analysis of the natural FXI mutation Gly155Glu provides some information. Gly155Glu is a rare example of a mutation causing FXI deficiency.72,73 Gly155 is located at the center of a loop connecting the β5 and β6 strands of A2. The Gly155 main chain nitrogen forms a hydrogen bond with the carbonyl group of Thr152 in a type I β-turn (Figure 4A). Gly155 points toward the center of a cavity in the apple domain disk and lies in a positively charged channel on the surface of A2 pointing away from the protease domain (Figure 4B).70 Recombinant FXI-Glu155 binds HK with approximately 10-fold lower affinity than wild-type FXI in a solid phase assay (D.G., unpublished observation, June 2008). The loop containing Gly155 forms hydrogen bonds with a second loop spanning residues 103 to 105 (Figure 4A) that contains the FXI mutation Gly104Asp and borders a pocket in the A2 domain at the end of the charged channel (Figure 4B). A Gly104Arg mutation in the PK A2 domain associated with CRM1 PK deficiency also causes decreased HK binding.74

FXI zymogen activation

In vitro, known plasma activators of FXI include FXIIa,4 α-thrombin,13,14,75 the prothrombin activation intermediate meizothrombin,76 and FXIa (autoactivation).13,15 all of which cleave FXI at the Arg369-Ile370 bond.5,6 Although FXIIa activates FXI in the cascade/waterfall model (Figure 1), the absence of a bleeding disorder in FXII-deficient patients indicates that alternative mechanisms for FXI activation exist. This is demonstrated clearly by the work of Sprock et al using mice lacking FIX, FXI, or FXII on a background of low tissue factor (TF) expression.77 Low TF mice have a severe bleeding disorder but are viable.78 Superimposing FIX or FXI deficiency on the low TF background results in death in utero from bleeding, implying FIX activation by FXIa is required for viability in the setting of a hemostatic system crippled by low

Figure 3. The structure of zymogen FXI. Topology diagram of dimeric FXI viewed from 2 perspectives rotated 90 degrees. The catalytic domain is in white. Sites of residues implicated in ligand binding are red for thrombin (T), green for HK, black for GPIb, blue for heparin sites (H1 and H2), and orange for FIX. Positions for the activation loop (AL) cleavage site (Arg360-Ile370) residue Ile370 and active site (AS) residues Ser557, Asp462, and His413 are shown in purple.
TF levels. Mice with deficiency of FXII and low TF, on the other hand, are viable and phenotypically similar to low TF mice with normal FXII expression. Although these data confirm the impression that FXIIa is not required for FXI activation, they should not be interpreted as indicating that FXIIa does not activate FXI in vivo. Indeed, FXIIa-mediated activation of FXI appears to play a central role in formation of pathologic thrombi in murine thrombosis models.35,38

Naito and Fujikawa13 and von dem Borne et al15 were the first to present results indicating that FXI is activated by thrombin in plasma. In purified protein systems, FXI activation by thrombin is enhanced by charged substances, such as dextran sulfate or heparin,13,14 but the relevance to activation in vivo is not clear. Thrombin interacts with most of its substrates through 2 electrostatics.2,79 Using a panel of thrombins with site-specific mutations, Yun et al showed that ABE I and ABE II are required for efficient FXI activation in the presence of dextran sulfate.80 Here ABE II interacts with dextran sulfate, whereas ABE I probably binds FXI. Recent experiments in plasma, however, did not identify a role for ABE I in FXI activation, as wild-type thrombin and thrombin with mutations in ABE I initiated FXI-dependent thrombin generation similarly.51

Available information indicates that thrombin binds to the A1 domain of FXI, with residues Glu66, Lys83, and Gln84 forming part of the binding site.2,7,77,75 These residues cluster near the interface with the A4 domain (Figures 2B, 3A) and are suitably positioned in proximity to the activation loop containing the Arg369-Ile370 cleavage site. The FXI residues involved in binding FXIIa have not been definitively determined, and the interaction may extend beyond a single apple domain surface. For example, although studies using peptide mimicry point to a binding site for FXIIa on the A4 domain,52 a monoclonal antibody that recognizes A2 selectively interferes with FXI activation by FXIIa.76,81

A role for the FXI dimer in promoting zymogen activation by thrombin in trans has been proposed.7 Here the activating protease would bind one subunit of the dimer and cleave Arg369-Ile370 on the other. Consistent with this, Wu et al used a series of recombinant monomeric FXI proteins in a purified system to show that the dimer is required for optimal FXI activation by thrombin, FXIIa, and FXIa.50 Some of these studies used dextran sulfate to facilitate FXI activation, which may not suitably model the physiologic environment. A second study with similar FXI monomers gave somewhat contradictory results, with monomers proving more susceptible than dimers to spontaneous autoactivation by FXIa, but here experiments were conducted in conditioned media (unpurified system).51

The term FXIa describes the protease form of FXI that has been cleaved at the Arg369-Ile370 peptide bonds on both subunits of the dimer. Recently, FXI activation by thrombin or FXIIa was shown to proceed through an intermediate with 1 activated subunit, referred to as 1/2-FXIa.51,83 The intermediate can be observed on nonreducing sodium dodecyl sulfate-polyacrylamide gels because FXI, 1/2-FXIa, and FXIa migrate at slightly different rates (Figure 5). 1/2-FXIa can be detected in plasma induced to undergo contact activation.53 Given that conversion of FXI to fully activated FXIa is a slow process, 1/2-FXIa may be a major species of activated FXI. The functional properties of this protease are discussed further in “Recognition and cleavage of FIX by FXIa.”

**FXIa protease domain structure and interactions with inhibitors**

There are no structures available for full-length FXIa or 1/2-FXIa, but structures for isolated FXIa catalytic domain in complex with inhibitors have been reported.40-42 Comparison with the zymogen structure reveals conformational changes expected with activation of a serine protease, including a 12Å movement of Ile370 (the N-terminus of the catalytic domain, Figure 6A) into the protease domain core. Trypsin-like proteases, including FXIa, cleave their substrates after basic amino acids (arginine in the case of FXIa) that are designated the P1 residue. The site on the protease that interacts with the substrate P1 residue is the S1 site. Changes in the catalytic domain that occur during FXI activation result in a salt bridge forming between the Ile370 N-terminal amine and the Asp554 side chain. This allows formation of the S1 site that will be occupied by the side chain of the P1 arginine from a substrate or inhibitor. A distinct conformational change is also observed in loops on the side of the catalytic domain opposite the active site cleft. This involves unraveling of a short a-helix in the zymogen and a closing together of the loop containing Arg489 and a b-hairpin to fill a pocket left empty by removal of the A3 domain, which forms an interface with the base of the catalytic domain in the zymogen (Figure 6A.C). The potential importance of this conformational change in the catalytic domain is not clear and requires further investigation.

![Figure 4. FXI-binding site for HK.](image)

(A) The relative positions of Gly104 and Gly155 in the A2 domain are shown. Gly155 is involved in a tight b-turn forming a hydrogen bond with Thr152. The compact hydrogen bond network extends through Thr152 and Thr158 to form contacts to the main chain through residues Lys103 and Ile105. Changes at Gly104 or Gly155 will probably disrupt this network. (B) Charged surface representation of the underside of the FXI apple domain disk, showing positions of Gly104 and Gly155. These residues are required for proper formation of a charged channel that is a probable binding site for HK. The dotted line represents the predicted binding site of HK that terminates in a pocket at the base of the A2 domain.

![Figure 5. FXI activation.](image)

Each FXI subunit is activated by cleavage between Arg369 and Ile370. FXI (0-hour time point) migrates slightly faster than FXIa (top band, 6 hours) on nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Activation of FXI (schematic at left) by a-thrombin (shown) or FXIIa proceeds through an intermediate with 1 cleaved subunit (center), designated 1/2-FXIa. Subsequent conversion of 1/2-FXIa to fully activated FXIa (right) appears to be a slower process. In the diagrams, the A1, A2, A3, and A4 domains are shown in light blue, cyan, orange, and yellow, respectively, and the catalytic domains (CAT) are in white. Circles represent unactivated catalytic domains; three-fourths circles, activated catalytic domains. In these diagrams, the catalytic domain moves relative to the apple domain disk after cleavage of Arg369-Ile370, exposing a surface on A3 thought to contain a FIX-binding site.
Recognition and cleavage of FIX by FXIa

FXIa’s major substrate, FIX, has an elongated structure that includes (from the N-terminus) a calcium-binding Gla domain, 2 EGF repeats, a 55-amino acid activation peptide, and a C-terminal serine protease domain (Figure 7A).90 Conversion of FIX to the protease FXaβ requires cleavages of the Arg145-Ala146 and Arg180-Val181 bonds releasing the activation peptide. When FIX is activated by the factor VIIa (FVIIa)/TF complex (Figure 1), there is preference for initial cleavage after Arg145, resulting in accumulation of the intermediate FIXα, followed by cleavage after Arg180 (Figure 7B).21-23 In contrast, relatively little or no FIXα accumulates during FIX activation by FXIa (Figure 7C).50,94-96 More than 1 mechanism is compatible with this observation. Cleavage of the FIX activation sites by FXIa could be sequential, with the rate of the second cleavage greater than the first. Indeed, FXIa, like FVIIa/TF, cleaves FIX initially at the Arg145-Ala146 bond.83,96 Alternatively, the protease may cleave sequentially but not release the intermediate before the second cleavage. This is consistent with data showing that cleavage rates of FIX and FIXα by FXIa are roughly similar (ie, Arg180-Val181 is not cleaved more rapidly than Arg145-Ala146).95 The possibility that FXIa requires its 2 catalytic domains to cleave the FIX activation sites simultaneously has been refuted, as 1/2-FXIα93 and monomeric variants of FXIα50 activate FIX similarly to dimeric FXIa.

Initial recognition of FIX involves exosites on FXIa that are distinct from the catalytic active site.96,98 FIX does not bind FXI,89 indicating that conformational changes during conversion of FXI to FXIa expose the exosites. An antibody that recognizes an epitope on the FXIa apple domain disk competitively inhibited FIX activation by FXIa.98 Subsequent work with recombinant proteins localized a FIX-binding site to the A3 domain (Figures 2B, 3).62,63 A critical residue for FIX activation is Arg184,63 located near the N-terminus of A3. An Arg184Gly mutation was recently reported in a CRM+ FXI-deficient patient, and recombinant FIXα with this mutation activates FIX poorly.100 In thezymogen structure, Arg184 occupies a central location in a loop connecting A2 and A3 that is buried under the protease domain (Figure 6A,C). The long Arg side chain to substrate recognition is discussed in “Recognition and cleavage of FIX by FXIa.”

Activated FXIa is subject to negative regulation by plasma serpins, including antithrombin, C1-inhibitor, protease nexin 1, and protein Z–dependent protease inhibitor.59-61,84-87 Basic amino acids in the autolysis loop of the catalytic domain (Arg504, Lys505, Arg507, and Lys509) are determinants of serpin specificity.87 Heparin enhances serpin-mediated FXIa inhibition through a mechanism involving heparin-binding sites on the A3 domain (Figures 2B, 3).58,59 and catalytic (Lys529, Arg530, Arg532, Lys536, and Lys540)61,62 domains (Figure 3). The A3-binding site facilitates inhibition through a template mechanism in which both protease and serpin bind to heparin.61 In contrast, heparin binding to the catalytic domain facilitates inhibition in a manner not completely explained by a template mechanism, and probably involves charge neutralization or an allosteric effect that overcomes repulsive interactions between the serpin and catalytic domain.61

FXIa is also regulated by protease nexin 2 (PN2).41,88,89 which is released from activated platelets.88 FXIa inhibition requires the Kunitz-type protease inhibitor (KPI) domain of PN2. A crystal structure of the FXI catalytic domain complex with PN2-KPI shows that the inhibitor has a characteristic disulfide-stabilized double-loop structure that fits into the FXIa active site, with the P1 arginine occupying the S1-binding pocket (Figure 6B).41
chain extends such that the guanidinium group fills the small pocket formed by the interface between the alpha domains and catalytic domain. Here, Arg184 interacts with 3 residues: 2 from the catalytic domain (Asp488, Asn566) and 1 from the A3 domain (Ser268; Figure 6D).

Arg184 is conserved in FXI across species but is not present in PK, consistent with its role in interactions with FIX. The Arg184 side chain must be exposed in FXIa by conformational changes that occur after cleavage of the Arg369-Ile370 bond in the activation loop. Full-length FXI and FXIa have been analyzed by small-angle x-ray scattering and electron microscopy, which revealed a change in the shape of the molecule on activation, consistent with a shift in the position of the apple domain relative to the catalytic domain. This could break interactions between Arg184 and Asp488, Asn566, and Ser268 (Figure 6D). Arg184 may, therefore, be part of a switch that holds FXI in an inactive conformation in the zymogen and, when the switch is thrown, facilitates engagement of FIX.

It appears that residues in the FIX Gla domain are required for binding to FXIa, explaining the enhancing effect of calcium ions on the reaction. There is also evidence that it is the Gla domain that binds to the A3 domain. Interestingly, loss of the A3 exosite has a more deleterious effect on cleavage of FIX at Arg180-Val181 than at Arg145-Ala146. This suggests that the A3 exosite primarily facilitates cleavage of FIX to FIXaβ and that a distinct exosite is involved in the initial interaction with FIX that facilitates conversion to FIXα. Recent work by Sinha et al indicates that this exosite is located on the FXIa catalytic domain. Interestingly, unlike binding to the A3 exosite, the interaction with the catalytic domain exosite does not require calcium, suggesting that the interaction does not involve the FIX Gla domain.

**FXI interactions with platelet receptors**

Although platelets do not appear to enhance FXI activation in a static system, there is growing evidence that platelets affect FXI and FXIa behavior under flow. When human blood lacking FXII activity is perfused over collagen at arterial shear rates, platelets deposit in aggregates and fibrin strands form. Addition of an antibody that blocks FXIa to the site of fibrin formation, as fibrin does not form when blood lacking FXII activity is perfused over collagen.

FXI lacks the Gla domain that facilitates binding of vitamin K-dependent coagulation proteases to phospholipid surfaces. Yet, there is solid evidence that FXI binds to platelets. Greengard et al measured approximately 1500 binding sites for FXI per activated platelet. Subsequent work determined that platelet GP1b mediates binding and that FXI competes with von Willebrand factor, but not thrombin, for binding to GP1b. Optimal binding of FXI to platelets requires residues in the FXI A3 domain (Figures 2B, 3), the N-terminal domain of the GP1bα chain, HK, and zinc ions. The A3 residues involved, Ser248 and Arg250, are near to, but distinct from, the A3 heparin-binding site. A CRM+ missense mutation in the A3 domain, Ser248Asn, was identified in a family with a bleeding disorder. FXI-Ser248 binds platelets with 5-fold reduced affinity compared with wild-type FXI, and other Ser248 substitutions (Gln or Ala) showed similar defects. Figure 8 shows the local environment around Ser248. The overall structure of the A3 domain is probably not affected significantly by the Ser248Asn mutation, as FXI-Asn248 is (1) secreted, (2) activated by FXIa, and (3) activates FIX similar to wild-type FXIa. Cumulatively, the findings make it probable that Ser248 and Arg250 are central to a site for interactions with platelet GP1b.

FXI also binds to platelets (~250 sites per platelet) but does so by a mechanism that appears to differ from that of FXI. FXIa binding to platelets does not require the A3 domain or HK, and involves residues in the catalytic domain between Cys527 and Cys542 in the vicinity of the heparin-binding site (Figure 3). The binding site on platelets for FXIa has not been identified but appears to be distinct from that of FXI, as FXI does not compete with FXIa for binding.

The observation that each platelet contains 1500 FXI-binding sites was somewhat perplexing, considering that there are approximately 25 000 copies of GP1b per platelet. New data from White-Adams et al may offer a solution to this conundrum; this group reported that FXI binds to a platelet receptor for apolipoprotein E called ApoER2 or LRP8. FXI binds to soluble ApoER2 with an affinity of approximately 60nM. Human and murine platelets bind and spread on glass coated with FXI or fibrinogen. Binding to FXI, but not fibrinogen, was abrogated by receptor-associated protein, which blocks ligand binding to members of the low-density lipoprotein receptor family. Platelet binding to FXI was also blocked by soluble ApoER2, and platelets from ApoER2-deficient mice did not bind FXI. Under conditions of physiologic shear, soluble ApoER2 or its low-density lipoprotein-binding domain prevented platelet binding to immobilized FXI.

There are an estimated 2000 copies of ApoER2 per platelet, which agrees well with the number of FXI-binding sites, suggesting that FXI binding involves both receptors. Consistent with this, antibodies to GP1b block platelet binding to immobilized FXI under physiologic shear. It is not clear whether the platelet-binding site on the FXI A3 domain is required for interactions with ApoER2 or whether a different site is involved.

**Future directions**

Several important aspects of FXI pathophysiology require additional study. Over the past 50 years, many investigators have

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**Figure 8.** Platelet-binding site on FXI. The locations of 2 residues (Ser248 and Arg250 in black), that probably form a GP1b-platelet-binding site on the FXI A3 domain (orange), are shown relative to residues that form the heparin-binding site (Ly252, Lys253, and Lys255 in blue). Ser248 forms hydrogen bonds with Asp194 and Thr249, which are probably disrupted in the hereditary FXI mutation Ser248Gln. The adjacent A2 domain is shown in light blue. The position of an N-linked glycan moiety attached to residue Asn108 of the A2 domain is shown in yellow and red.
commented on the weak correlation between bleeding and FXI activity measured by conventional assays. This is reflected in 2 studies involving 49 FXI-deficient kindreds (242 persons), which determined that severity of bleeding was similar in patients with severe and mild deficiency. In contrast, work with patients primarily with the Glu117Stop and/or Phe283Leu mutations showed bleeding risk to be much greater in severe than mild deficiency (odds ratio = 13.0 and 2.6, respectively). Diverse factors could be contributing to this disagreement, including differences in clinical criteria for bleeding, variation in genetic backgrounds or FXI mutations, or coinheritance of other bleeding disorders. That being said, some patients with severe FXI deficiency do not bleed abnormally, even with trauma to tissues that typically cause problems for FXI-deficient patients. We suspect that the manner in which we measure FXI activity in clinical laboratories does not accurately assess the protein’s contribution to hemostasis.

FXI-deficient plasma exhibits a prolonged aPTT clotting time, and FXI deficiency is currently defined as low activity in an aPTT-based assay in which patient and FXI-deficient plasma are mixed. However, the aPTT only requires FXI to function as a component of contact activation initiated fibrin formation, and does not assess FXI functions such as feedback activation by thrombin, inhibition of fibrinolysis, and interactions with platelets. The FXI Ser248Asn variant illustrates the limitations of the aPTT in assessing FXI function. This mutation is associated with bleeding and defective FXI binding to platelets but does not affect the aPTT assay, which does not contain platelets. Assays that capture the importance of FXI to sustained (rather than initial) thrombin generation, probably in platelet-rich plasma under flow, will be required to address the deficiencies of the aPTT. Allen et al made the interesting observation that the contribution of FXI to thrombin generation varied substantially when platelets from different normal donors were tested in a cell-based model of TF-initiated coagulation. Platelets, therefore, may turn out to be key for accurately assessing FXI function.

As presented in this review, FXI has features that distinguish it from vitamin K–dependent coagulation proteases, making it difficult to extrapolate from the lessons learned from the large body of work on these enzymes to predict structure-function relationships for FXI. There remain many important unanswered questions regarding FXI and FXIa from a structural and enzymologic perspective. Areas of active investigation include determining the functional significance of the dimeric structure of FXI/FXIa, elucidating the manner in which FXI/FXIa engages platelets, and identifying the functional consequences of platelet binding.

New evidence indicates that the dimeric structure of FXI facilitates its activation by FXIla and thrombin. However, the FXI homolog PK (a monomer) is activated rapidly by FXIla, as is a monomeric FXI chimera containing the PK A4 domain. It will be important to determine whether dimerization is critical for FXI activation under physiologic conditions, probably using flow-based systems, or perhaps in vivo.

It has been proposed that one subunit of a FXIla dimer could bind to a platelet receptor, leaving the other free to interact with the substrate FIX. An intriguing possibility that remains to be tested is that 1/2-FXIa binds to GP1b, or perhaps ApoER2, through the A3 domain of its unactivated subunit while binding to FIX through A3 on the activated subunit. Note that in Figure 3B the A3 domains point in opposite directions from the longitudinal plane of the molecule. As FXI lacks a Gla domain, the dimeric structure may represent an alternative solution to the problem of tethering the protease to a surface at a wound site while allowing interaction with its substrate. Such a hypothesis is also best tested in flow-based systems, where the FXI-platelet interaction is probably not important to protease function.

Recent studies associating FXIa activity and pathologic coagulation in humans and animal models have stimulated considerable interest in this protease as a target for clinical intervention, with potential advantages over targeting thrombin or FXa in terms of side effects and therapeutic window. Work on FXI structure is at an early stage, compared with the vitamin K-dependent coagulation factors, with crystal structural data becoming available only within the past 5 years. Structures defining full-length FXIa and ligand complexes with substrate, receptors, and activating enzymes will be required to completely understand the molecular basis of processes such as zymogen activation, platelet binding, and substrate cleavage. This will be critical to understanding the role of this protease in disease processes and to the development of specific inhibitors for clinical purposes.

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