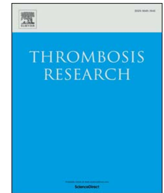
Thrombosis Research 161 (2018) 94–105

Contents lists available at ScienceDirect

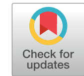
Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres



Review Article

An update on factor XI structure and function



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ARTICLE INFO

Keywords:
Factor XI
Factor XIa
Prekallikrein
Factor XIIa
Factor IX

ABSTRACT

Factor XI (FXI) is the zymogen of a plasma protease, factor XIa (FXIa), that contributes to thrombin generation during blood coagulation by proteolytic activation of several coagulation factors, most notably factor IX (FIX). FXI is a homolog of prekallikrein (PK), a component of the plasma kallikrein-kinin system. While sharing structural and functional features with PK, FXI has undergone adaptive changes that allow it to contribute to blood coagulation. Here we review current understanding of the biology and enzymology of FXI, with an emphasis on structural features of the protein as they relate to protease function.

1. Introduction

Factor XI (FXI) is the zymogen of the coagulation protease factor XIa (FXIa) [1,2]. In humans, the protein circulates in plasma at a concentration of ~30 nM (15–45 nM) almost entirely as a non-covalent complex with the glycoprotein high molecular weight kininogen (HK) [3–5]. FXI is unique among coagulation proteases in that it is a dimer of identical 80 kDa subunits [6–9]. It was first identified by Robert Rosenthal and his colleagues in 1953 as a plasma constituent missing in members of a family with a mild trauma-induced bleeding disorder, and was originally called plasma thromboplastin antecedent (PTA) [10,11]. In the past, the autosomal bleeding disorder associated with FXI deficiency has been referred to as Rosenthal syndrome, PTA deficiency or hemophilia C to distinguish it from deficiencies of factor VIII [hemophilia A] and factor IX [hemophilia B].

In the original cascade/waterfall hypotheses of coagulation, FXI is converted to FXIa by the protease factor XIIa (FXIIa) [12,13]. FXIa then promotes clotting by converting factor IX (FIX) to the protease FIXaβ. However, the absence of abnormal bleeding associated with deficiency of factor XII (FXII, the precursor of FXIIa) indicates this is not an important mechanism for stopping bleeding at a wound site (hemostasis) [14]. Furthermore, the mild bleeding disorder associated with FXI deficiency suggests FXI serves a supportive role in hemostasis, rather than a major role in initiating clot formation [15–17]. The diagram in Fig. 1 is a model depicting relationships between FXI and other plasma components that we currently use in our laboratories [18,19]. Here, FXI is a bridge between tissue factor-initiated thrombin generation (**Left Panel**) and the kallikrein-kinin system (KKS), a group of proteins involved in several host-defense and homeostatic processes (**Right Panel**) [20]. FXI can support thrombin generation independent of the KKS, can function as a substrate and protease within the KKS, or serve as a bidirectional interface that permits the two systems to influence each other. The role of FXI as it relates to these systems has been covered in several recent reviews [16,19–22]. Here, we discuss current understanding of FXI protein structure, molecular biology, and enzymology.

2. Evolutionary history

Homologs for components of the thrombin generation mechanism shown in the left panel of Fig. 1 are found in all but the most primitive vertebrate organisms [23–25]. Genes for KKS proteins (FXII, the protease precursor prekallikrein [PK] and the cofactor HK) are not present in the genomes of zebrafish or pufferfish, but are found in amphibians, reptiles and mammals, suggesting an adaptation to terrestrial environments [24–26]. The gene for FXI arose through duplication of the PK gene early in mammalian evolution or in a reptilian ancestor of mammals [26]. Originally, it was thought that the genome of the primitive egg-laying mammal (monotreme) the duck-billed platypus had a single gene for a PK-like protein [26], however, subsequent work identified sequences corresponding to separate PK and FXI genes in this species (M. Ponczek, personal communication).

cDNAs for PK [27,28] and FXI [7,8] encode proteins with four 90 to

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http://dx.doi.org/10.1016/j.thromres.2017.10.008
Received 21 September 2017; Received in revised form 4 October 2017; Accepted 9 October 2017
Available online 10 October 2017
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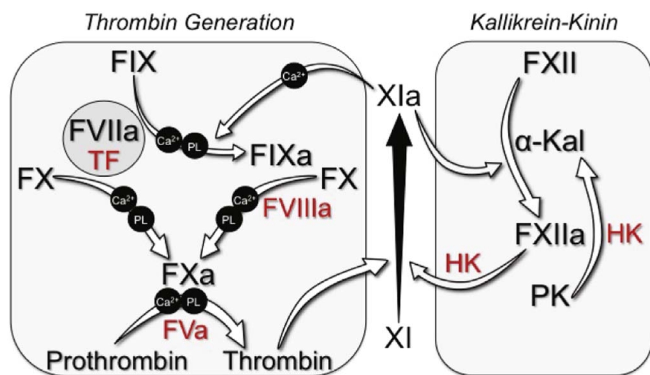


Fig. 1. Factor XI, thrombin generation, and the kallikrein-kinin system (KKS). Proteolytic reactions required for thrombin generation at an injury site are shown in the grey box on the left, while proteolytic reactions involving the KKS are in the box on the right. Protease zymogens are indicated in black, and their active forms by a lower case "a". Cofactors are in red. Requirements for calcium ions (Ca2+) or phospholipid (PL) are indicated. Thrombin generation is initiated by the factor VIIa/tissue factor (TF) complex, which activates factors (F) X and IX. Activated FX (FXa) converts prothrombin to thrombin. Thrombin generated early in coagulation converts FXI to FXIa, which sustains thrombin production through FIX activation. Note that FXI activation during thrombin generation does not require FXIIa. In the KKS, artificial or abnormal surfaces facilitate FXII auto-activation. FXIIa converts prekallikrein (PK) to α-kallikrein, which activates additional FXII and cleaves high molecular–weight kininogen (HK), liberating bradykinin (BK). The KKS can promote thrombin generation through FXIIa-mediated activation of FXI. There is evidence that FXIa, in turn, can activate FXII. In plasma, PK and FXI circulate as complexes with HK, which may serve as a cofactor for PK and FXI activation. FXI may act as a bidirectional bridge, allowing the thrombin generation system and KKS to influence each other. Image modified from Bane et al. [18] and Gailani et al. [19] with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

91 amino acid repeats called apple domains (or PAN domains for Plasminogen-Apple-Nematode) that are homologs of the N-terminal domains of plasminogen and hepatocyte growth factor [29]. While PAN domains are present in genes from a variety of organisms, no other proteins with four apple domains in the configuration seen in PK and FXI have been identified. Both proteins contain a C-terminal trypsin-like catalytic (protease) domain. The diagram in Fig. 2 shows the amino acid sequence, disulfide bonds, and domain organization of human FXI as described by McMullen and co-workers [8]. PK is similarly organized [28]. The homology with PK has facilitated studying FXI structure-function relationships.

3. Factor XI synthesis

The genes for FXI and PK are located within ~5 kilobases of each other on the distal end of the long arm of chromosome 4 (4q35) in humans, and are organized similarly (15 exons, exon 2 signal peptide, exons 3–10 apple domains, exon 11–15 protease domain) [30–32]. Like other coagulation protease precursors, plasma FXI is synthesized primarily in hepatocytes [33–35]. Normal expression of FXI is controlled by the transcription factor hepatocyte nuclear factor-4α (HNF-4α) [32]. In humans, FXI is also expressed in the Islets of Langerhans in the pancreas, and in renal tubule cells [36]. Expression in mice appears to be largely confined to the liver [35].

There is inconsistent literature regarding whether or not human platelets contain and/or express FXI, and the form the protein takes [37,38]. Several groups identified FXI mRNA identical to that found in liver in human platelets and several megakaryocytic cell lines [39–41]. Recently, Zucker et al. presented data showing FXI pre-mRNA in human platelets that is spliced to a mature form on platelet activation [41]. The activated platelet then translates the message, producing a protein that migrates similarly to plasma FXI on SDS-PAGE and is recognized by antibodies to human FXI. A similar process has been reported for platelet expression of interleukin 1β, tissue factor, and cyclooxygenase 2 [42]. The relative importance of FXI synthesized or carried by platelets to that of plasma FXI is not known.

4. Factor XI structure

Papagrigoriou et al. reported a structure for zymogen human FXI in 2006 [9]. Each apple domain consists of seven β-strands running antiparallel to each other that support a single α-helix (Fig. 3A). Three internal disulfide bonds constrain the apple domain structure. The apple domains (A1 to A4 from the N-terminus) form a planar disk-like structure ~60 Å wide, with A1 and A2 running anti-parallel to A3 and A4, separated by a 180° turn in the polypeptide chain (Fig. 3B). The apple domain disk has been likened to a "saucer" on which the protease domain (the cup) rests (Fig. 3C). The identical subunits of the FXI dimer interface through the A4 domains, with Cys^{321} forming an interchain disulfide bond (Fig. 3D). The apple domain disks are inclined at a 70° angle relative to each other creating an inverted V-shape. The two A2 domains are separated furthest from each other, while the A3 and A1 domains on one subunit are located close to the A1 and A3 domains, respectively, of the other subunit.

Each FXI subunit is converted to an active protease by cleavage after Arg^{369} (Fig. 2) [1,7,8]. While a crystal structure is not available for FXIa, studies using low-resolution electron microscopy and small angle X-ray scattering reveal large-scale conformational changes accompany conversion of FXI to FXIa [43]. As discussed below, FXIa activation of FIX requires FIX to bind to an exosite on the FXIa A3 domain that is not available in FXI [44]. This implies a significant conformational change on activation that unmasks the FIX binding site. This is simulated in the images in Fig. 4A to C. The interaction is discussed in more detail in the section on Factor XIa Activation of Factor IX.

5. The factor XI dimer

The A4 domains of the two subunits in the FXI dimer form an 886 $Å^2$ interface, with the β-sheets packed against each other (Fig. 4D) [9,47]. Cys321 [7], located on a loop projecting away from the main body of A4, forms an interchain disulfide bond [45]. Leu^{284}, Ile^{290} and Tyr^{329} form the hydrophobic core of the interface [9,46,47], and salt bridges between Lys^{331} and Glu^{287} of opposite subunits contribute to dimer stability [9]. Replacing Cys^{321} does not disrupt the FXI dimer, showing the importance of non-covalent interactions (Fig. 4E) [45–51]. The structure of FXI and mutagenesis data demonstrate the central role of the A4 domain in dimer formation, however, A4 is not the only apple domain driving dimerization. In PK, a monomeric protein, Cys^{321} forms an intra-domain disulfide bond with Cys^{326}, a residue not present in FXI [28]. Removal of PK Cys^{326} to leave Cys^{321} unpaired does not promote PK dimerization [50]. However, FXI in which the A4 domain is replaced with a PK A4 domain lacking Cys^{326} (leaving Cys^{321} unpaired) readily dimerizes despite the absence of the FXI A4 domain [50]. Here the A3 and A2 domains, while perhaps not directly contributing to the interface, are somehow involved in promoting dimerization in a manner not made obvious by the available crystal structure.

The FXI dimer is conserved across species, implying functional importance [51]. Critical components of the A4 interface, and an unpaired Cys^{321}, are present in all species for which mRNA sequences are available, with the exception of the rabbit, which has histidine replacing Cys^{321} [52]. However, gel filtration studies show that rabbit FXI, like recombinant human FXI lacking Cys^{321}, is a non-covalently associated homodimer. A function for the FXI dimer is not clearly established. Monomeric forms of FXI can be generated by replacing Cys^{321} and either Leu^{298} or Ile^{290} with alanine (Fig. 4E) [46,47,53,54]. These proteins display activity similar to dimeric FXI when used to supplement FXI-deficient plasma in activated partial thromboplastin time (aPTT) clotting assays. In our hands they are also similar to dimeric FXI/XIa in terms of ability to undergo autoactivation and activation by thrombin, and to activate FIX [53,55–57]. Unlike dimeric FXI, they fail

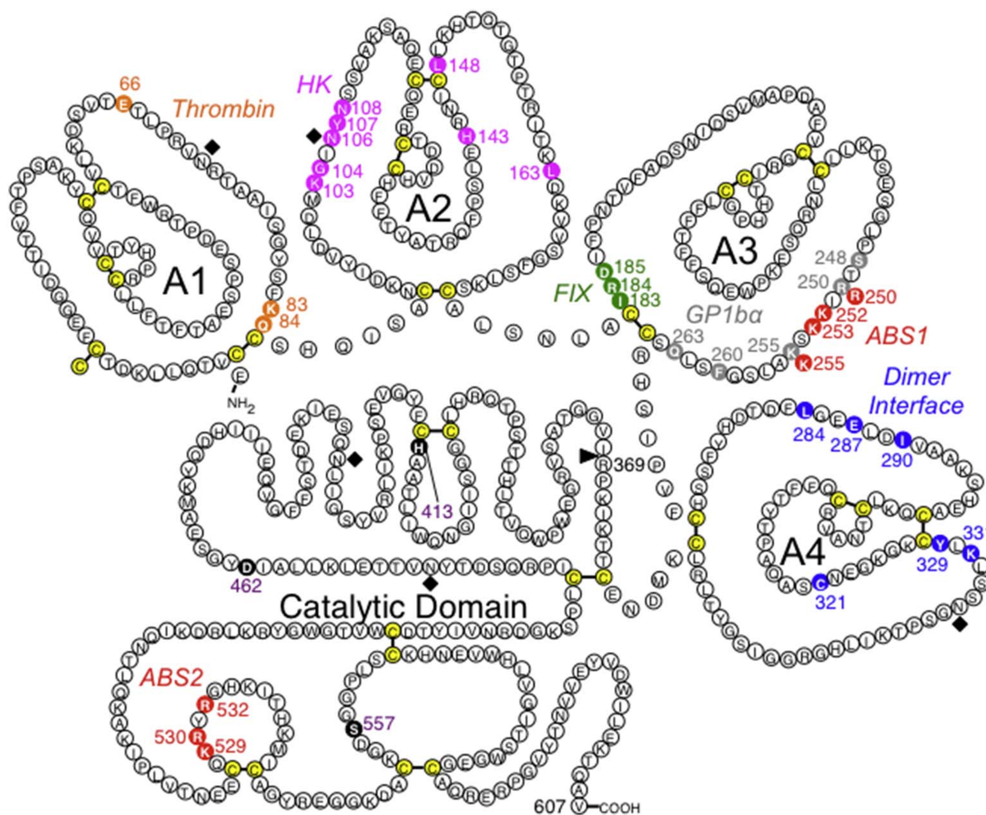


Fig. 2. Human Factor XI amino acid sequence, disulfide bond structure, and domain organization. Factor XI (FXI) is comprised of four apple domains (A1 to A4) and a trypsin-like Catalytic Domain. In this diagram, each amino acid is represented by a circle, with cysteine residues indicated in yellow. Conversion of FXI to FXIa involves cleavage after Arg^{369} (arrow). Residues identified as having specific functional importance are indicated by various colors. The catalytic triad required for proteolytic activity is indicated in black (His^{413}, Asp^{462}, Ser^{557}). Resides involved in interactions with thrombin (orange - Glu^{66}, Lys^{83}, Gln^{84}), HK (magenta – Lys^{103}, Gly^{104}, Asn^{106}, Tyr^{107}, Asn^{108}, His^{143}, Leu^{148}, and Leu^{163}), factor IX (green - Ile^{183}, Arg^{184}, Asp^{185}), the platelet GP1b receptor (grey - Ser^{248}, Arg^{250}, Lys^{255}, Phe^{260}, and Gln^{263}), and polyanions (red – ABS1 Arg^{250}, Lys^{252}, Lys^{253}, and Lys^{255}; ABS2 Lys^{529}, Arg^{530}, and Arg^{531}) are shown. The Abbreviations ABS1 and ABS2 stand for Anion binding Site 1 and 2, respectively. Residues in blue (Leu^{284}, $Glue^{287}$, Ile^{290}, Tyr^{329} and Lys^{331}) are components of the interface between the two subunits of the FXI dimer, with Cys^{321} forming a disulfide bond between the dimer subunits. Black diamonds indicate locations of putative N-linked glycosylation sites. After McMullen et al. [8], with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to reconstitute FXI-deficient mice in thrombosis models, consistent with an important role for the dimer *in vivo* [53]. At low FXIIa concentrations, FXI monomer is activated more slowly than FXI dimer [46,53]. This may explain the loss of the prothrombotic effect in mice, but would not appear relevant for the proposed role of FXI in hemostasis. We are investigating the possibility that one FXI subunit is required for interaction with a surface such as a platelet, while the other activated subunit engages FIX [57]. Such interactions, while not required for protease function in static assays, may be important for protease function in flowing blood.

6. Factor XI post-translational modifications

Glycosylation accounts for ~5% of the molecular mass of human FXI (Fig. 5A) [58]. Each subunit has five consensus sites for *N*-glycosylation (Fig. 2, N72, N108, N335, N432, and N473) [7,8]. Meijers et al. reported in 1992 that each of these residues, with the exception of N335, is glycosylated [59]. Using mass spectrometry, Faid et al. confirmed that N72, N108, N432, and N473 are glycosylated on > 90% of FXI subunits [60], while N335 is not glycosylated. N335 is located near the dimer interface, and glycosylation at this location would probably interfere with dimerization. A noncanonical site (N145) is glycosylated in ~5% of subunits [60]. We replaced Asn residues at the putative *N*-glycosylation sites in FXI with alanine and, with the exception of residue 335, noted reductions in apparent molecular mass on SDS-PAGE consistent with loss of a glycosylation site (Fig. 5B, D. Sun, M-f Sun and D Gailani, unpublished data). The glycosylation mutants have normal enzymatic activity in plasma, and activity of plasma FXI is not altered by deglycosylation (D. Sun, M-f Sun and D Gailani, unpublished data). However, glycosylation may play an important role in protein stability in plasma *in vivo*. FXI plasma levels are often low in patients with defects in N-linked glycosylation [61]. We observed that the half-lives of recombinant FXI proteins infused into FXI-deficient mice vary widely depending on the cell line in which they were expressed (Q Cheng, M-f Sun, D Gailani, unpublished observation). The differences in the FXI preparations are likely due to variation in post-translational glycosylation, which may vary substantially between cell lines.

7. FXI anion-binding

Human FXI contains two clusters of basic amino acids, one on the A3 domain starting at Arg^{250} (R-I-K-K-S-K) [62,63] and the other on the protease domain 170-loop starting at Lys^{529} (K-R-Y-R) [64], that interact with polyanions such as heparin [62–64], polyphosphate [65–67] and nucleic acids [68] (Figs. 2 & 5C). We refer to these clusters as anion binding site (ABS) 1 and 2, respectively. ABS1 and ABS2 were first recognized as sites required for normal expression of heparin cofactor activity during FXIa inhibition by antithrombin [62–64], and were subsequently shown to mediate binding to polyphosphate chains that enhance FXI activation by thrombin and FXIIa, and promote FXI autoactivation [65,66]. On a polyanion, FXI/XIa and an inhibitor (antithrombin) or activator (thrombin or FXIIa) bind in proximity to each other to achieve maximum rates of inhibition/activation by a "template" mechanism characterized by bell-shaped dependences on polyanion concentration.

PK lacks an ABS on its A3 domain. While it has basic residues on its 170-loop that correspond to those of FXI ABS2 [27,28], the site does not appear to be required for polyanion binding [68]. PK binds more weakly than FXI to polyanions (Ivanov and Gailani, unpublished observation), even though polyanions enhance PK/kallikrein mediated reactions [68–71]. For PK, normal activation and activity require HK, which facilitates PK binding to the polyanion [20,68]. In contrast, HK has an inhibitory effect on FXI activation in the presence of polyanions, and cannot substitute for ABS1 and ABS2 to support polyanion-enhanced FXI activation [68]. These data suggest a fundamental difference in the manner in which FXI and PK interact with polyanions, and that HK may have an inhibitory regulatory role in FXI activation.

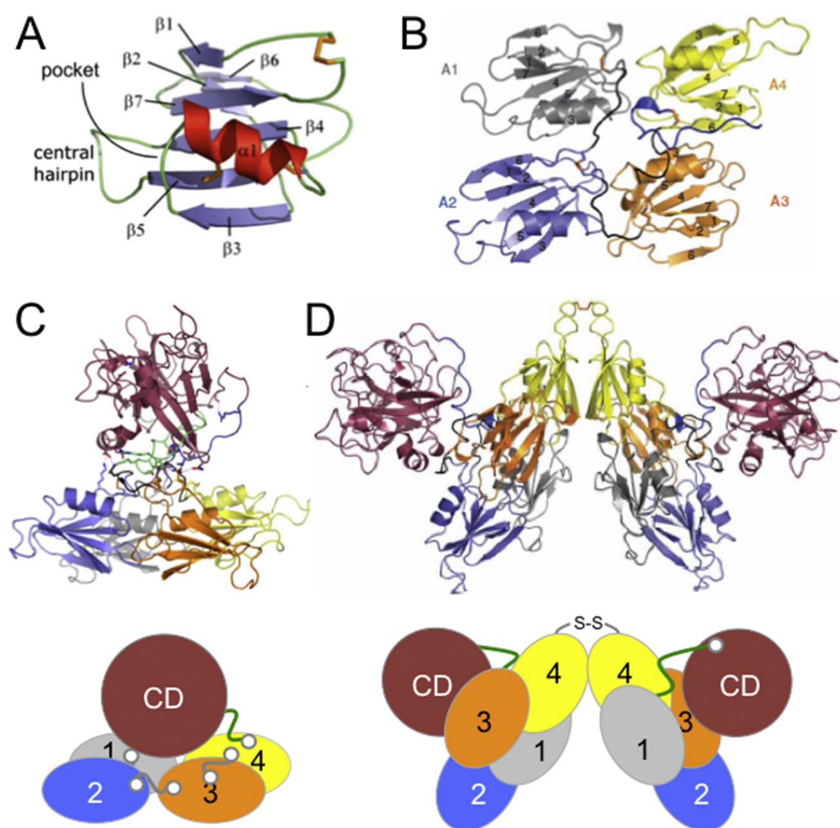


Fig. 3. Factor XI zymogen structure. (A) Ribbon diagram of the isolated FXI apple 1 domain from the crystal structure of the full length FXI zymogen (pdb:2F83). The α-helix is indicated in red and the β-sheet in blue. Disulfide bonds are in yellow. (B) Topology diagrams for the first, second, third, and fourth apple domains (A1, A2, A3, and A4) are shown in grey, blue, orange, and yellow, respectively. (C) Ribbon diagram and schematic of the FXI monomer with the catalytic domain (CD) colored maroon and the activation loop cleavage site residues Arg^{369}—Val^{370} colored green. Apple domains (numbered 1 to 4) are indicated by the colors described in panel B. (D) Ribbon diagram and schematic of the FXI dimer with the A4 domains of each subunit forming the dimer interface. The Cys^{321}—Cys^{321} bond at the top of the diagram covalently connects the subunits. From Papagrigoriou et al. [9]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

8. The FXI interaction with HK

Almost all FXI (3,4) and 75–90% of PK [4,72] circulates in complex with HK, a highly glycosylated 120-kDa 644 amino acid plasma protein that is organized into 6 domains (D1 to D6) [20,73]. The C-terminal D6 domain binds FXI and PK, with at least 58 amino acids (HK residues 556–613) required for normal FXI binding, and a 31 amino acid segment required for PK binding [74]. The physiologic importance of the PK-HK interaction seems clear as HK cleavage by α-kallikrein (activated PK) liberates the vasoactive peptide bradykinin from HK [20,21,73], and HK enhances PK activation by FXIIa [20,68,73,75,76]. The importance of the FXI-HK interaction is less clear. Although HK may facilitate FXI binding to surfaces such as platelets [77–80] and endothelial cells [81], we observed that HK tends to inhibit FXI activation in the presence of DNA or polyphosphate [68].

Studies with FXI/PK chimeras, individual apple domains, and peptide sequences derived from apple domains indicate the FXI A2 domain is required for HK binding [82,83]. Domains A1 and A4 may also contribute [82,83]. A major advance in understanding the FXI-HK interaction came with the determination of the crystal structure for FXI in complex with an eight amino acid peptide derived from the HK D6 domain called HKP [84]. The central region of D6 containing the sequence Asn-Pro-Ile-Ser-Asp-Phe-Pro-Asp (residues 583–590) binds to a pocket in the A2 domain β-sheet (Fig. 6A & B). The HK Asp-Phe-Pro motif is a major binding determinant, with Asp587 forming a key salt bridge with FXI Lys103, and Phe588 inserting into a hydrophobic pocket in FXI A2.

Fig. 6C & D shows the two HKP peptides binding to the A2 domains of the FXI dimer on the face of the apple domain disks opposite the protease domains. In Fig. 6C the top (northern) hemisphere contains the FXI protease domain (maroon), activation loop (green), and A4 domain (yellow), while the bottom (southern) hemisphere contains the A2 domains (light blue) with the HK-D6 peptides (purple). The A3 domains (orange) and A1 domains (grey) lie within the FXI equatorial region. The HK peptides face the dimer axis in an arrangement that would effectively localize HK in the A2 domain binding pockets in the same plane at a distance of 45 Å from each other (Fig. 6D). The FXI dimer interface is at an oblique angle so that bound HK peptides are oriented toward each other. The data suggest a FXI dimer binds up to two HK molecules. This is supported by gel filtration experiments, which reveal a 1:2 M ratio of FXI and HK resulting in a single ~400 kDa complex (Fig. 6E). Edman degradation analysis confirms an equimolar interaction between FXI subunits and HK polypeptides (*i.e.* two HK binding to each FXI dimer) [84]. HK has been reported to be a disulfide-linked circular monomer [85] or a dimer [86,87] by SDS-PAGE, however, gel filtration studies consistently indicate the protein is only a dimer [3,84,87]. Given this, it is possible that each FXI dimer actually associates with one HK dimer, as illustrated in Fig. 6F.

9. FXI interaction with laminin

Laminins are large heterotrimeric proteins that are required for normal formation of extracellular matrices. Laminins 411 and 511 are abundant constituents of the extracellular matrix of blood vessels and arteries. FXI binds to human recombinant laminin heterotrimers 111, 411 and 511 in a concentration-dependent manner with K_d values of 11.1 nM, 9.2 nM, 8.3 nM, respectively [84], and to laminin 111 purified from the native basement membrane of a murine sarcoma cell line (K_d 18.8 nM) [84]. It does not bind to triple helical collagens [84]. Laminins are now recognized as important contributors to thrombus formation, and their interactions with platelet integrins are well-characterized [88]. Laminin provides a surface that can promote plasma coagulation and thrombus formation through FXII and FXI [89]. HK and FXII have both been reported to bind laminin [90,91]. It is notable that the FXIa substrate FIX binds to basement membrane through an interaction with collagen IV [92,93]. The interaction between FXI and laminin may be a mechanism for co-localization of FXIa and its substrate FIX to promote thrombin generation on the extracellular matrix

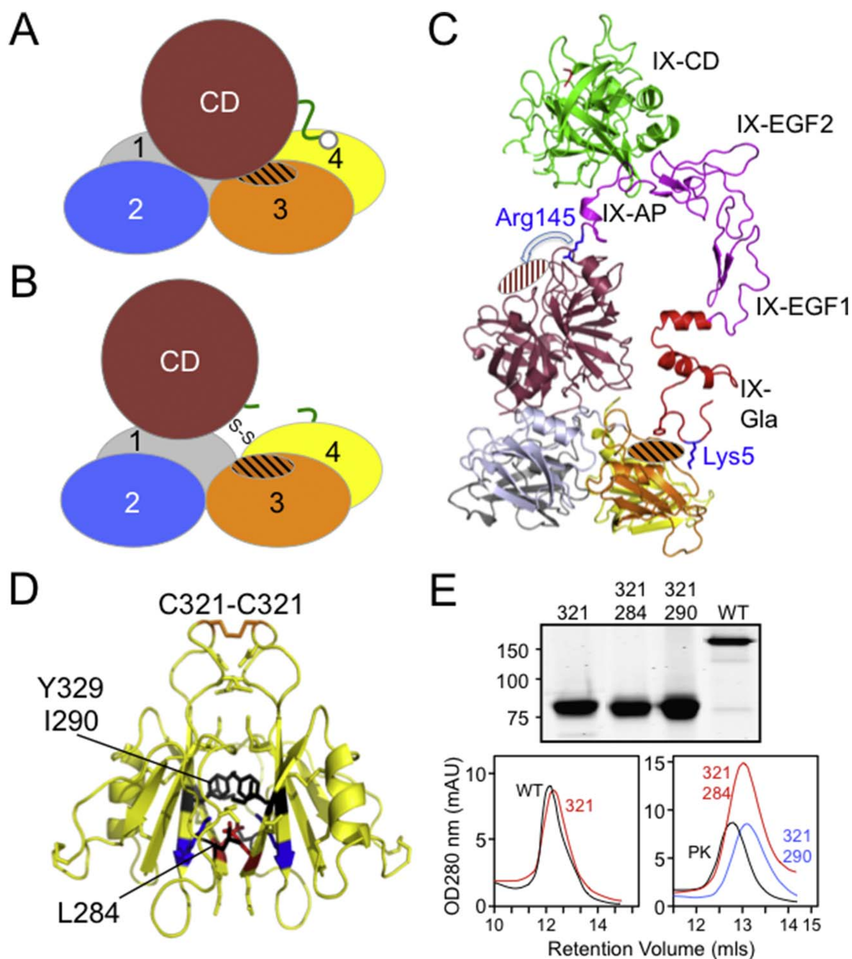


Fig. 4. Model of conversion of FXI to FXIa and the FXI dimer interface. (A) Cartoon of a FXI subunit depicting the catalytic domain (CD) covering a FIX-binding site (cross-hatched area) on the A3 domain. Apple domains are numbered 1 to 4. (B) Cartoon of a FXIa subunit showing the predicted conformational change accompanying cleavage of the Arg^{369}—Val^{370} bond that results in movement of the catalytic domain relative to the apple domain disk, and unmasking of the FIX binding site. (C) Topological diagram showing the FXI structure with the catalytic domain (maroon) displaced to reveal the FIX binding exosite in the A3 domain (orange cross-hatch). The FIXa crystal structure (pdb:1PFX) is shown as a ribbon diagram to illustrate the scale comparison of a vitamin-K dependent coagulation factor and FXI subunit. The FIX Gla domain (red) including residue Lys^{5} can contact the FXIa A3 domain exosite, while the EGF domains (magenta) project upwards to position the FIX activation peptide (AP) residue Arg^{145} in the area of the FXIa S1 pocket (maroon cross-hatched). The FIX protease domain is colored green. (D) FXI dimer interface. Shown are two FXI A4 domains forming the dimer interface. The Cys^{321}—Cys^{321} interchain disulfide bond is shown at the top in orange. Hydrophobic residues Leu^{284}, Ile^{290}, and Tyr^{329} are shown in black, and a salt bridge is formed between Lys^{331} (blue) and Glu^{287} (red). From Papagrigoriou et al. [9]. (E) FXI monomers. The top panel is a Coomassie Blue stained SDS-PAG showing non-reduced samples of wild type FXI (WT) and FXI species with Cy^{321} changed to serine (321). 284 and 290 indicate proteins that have also had Leu284 or Ile290 changed to alanine. The bottom images are elution profiles from size exclusion columns demonstrating that FXI with replacement of Cys^{321} alone elutes with FXI dimer (WT), while proteins with replacement of Cys^{321} and either Leu^{284} or Ile^{390} elute with the monomer prekallikrein (PK). From Geng et al. [53]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

after blood vessel injury.

10. Conversion of FXI to FXIa

Each FXI subunit may be converted to its active form by several forms of thrombin (α-thrombin, β-thrombin, γ-thrombin, and meizothrombin) [94–97], factor XIIa [20,53,71], or by FXIa (autoactivation) in the presence of polyanions [65,66,68,95]. Regardless of the activating protease, FXI activation requires cleavage of the Arg^{369}—Ile^{370} bond [7,8] (Fig. 2), creating a ~50 kDa heavy chain that contains the apple domains and a 30 kDa light chain that is the catalytic domain (Fig. 7A). The heavy and light chains are connected by a Cys^{362}—Cys^{482} disulfide bond (Fig. 2). The designation "FXIa" is used to describe FXI with both subunits cleaved after Arg^{369}. However, when FXI is activated by thrombin or FXIIa the first species formed has only one activated subunit [55,56]. We refer to this form as 1/2-FXIa. On non-reducing SDS-PAGE FXI, 1/2-FXIa, and FXIa migrate at slightly different rates relative to each other (Fig. 7B) [55]. 1/2-FXIa activates FIX by the same mechanism as FXIa with both subunits activated (discussed below). *In vitro*, conversion of FXI to 1/2-FXIa is considerably more rapid than conversion of 1/2-FXIa to FXIa, suggesting 1/2-FXIa may be a physiologically important species of activated FXI.

In aPTT assays FXIIa activates FXI [2,14]. In mouse and primate models, this reaction appears to contribute to thrombosis [51,71,98,99], however, it is unlikely to be required for hemostasis given the absence of a bleeding diathesis in FXII-deficient individuals [2,14]. Studies comparing FXI dimers and monomers suggest there may be two types of binding interactions between FXIIa and FXI; a high affinity interaction operating by a *trans*-mechanism in which FXIIa binds to one subunit of the FXI dimer while activating the other subunit, and a lower-affinity *cis*-activation in which FXIIa binds to and activates the same FXI subunit [46,53]. The FXI residues involved in FXIIa binding are not known. The interaction may involve more than one apple domain. While studies using peptide mimicry point to a binding site on the A4 domain [100], an antibody to A2 selectively interferes with FXI activation by factor XIIa [51].

While clinical observations [14] and some pre-clinical work with gene-deleted mice [101,102] support the premise that FXI contributes to hemostasis in a FXIIa-independent manner, the identity of the protease(s) that activate FXI *in vivo* are not established. Forms of thrombin are leading candidates. The X-Pro-Arg sequences preceding the FXI activation cleavage site in most mammals are typical thrombin cleavage sites, and differ from sequences found in PK [97]. FXI activation by thrombin is enhanced by polyphosphate [53,65,66], nucleic acids [68,103–105], dextran sulfate [95] and various glycosaminoglycans [106,107] through a template mechanism as described above. Thrombin anion binding exosite II facilitates thrombin binding to polyanions during FXI activation, but anion binding exosite I does not appear to be required for FXI activation [97]. There is limited data regarding a thrombin-binding site on FXI. A1 domain residues Glu^{66}, Lys^{83}, and Gln^{84} (Fig. 2) have been implicated [9,108,109]. These residues cluster near the interface with the A4 domain, and are in proximity to the activation loop containing the Arg^{369}—Ile^{370} cleavage site.

The structures of the thrombin and FXIIa protease domains are quite different. Thrombin uses its positively charged anion binding exosites to interact with a variety of substrates [110], while the FXIIa protease domain has a net negative charge and exosites that may bind FXI have not been identified [111,112]. FXI has an overall net positive charge, and charge complementarity with FXIIa may explain why it can activate

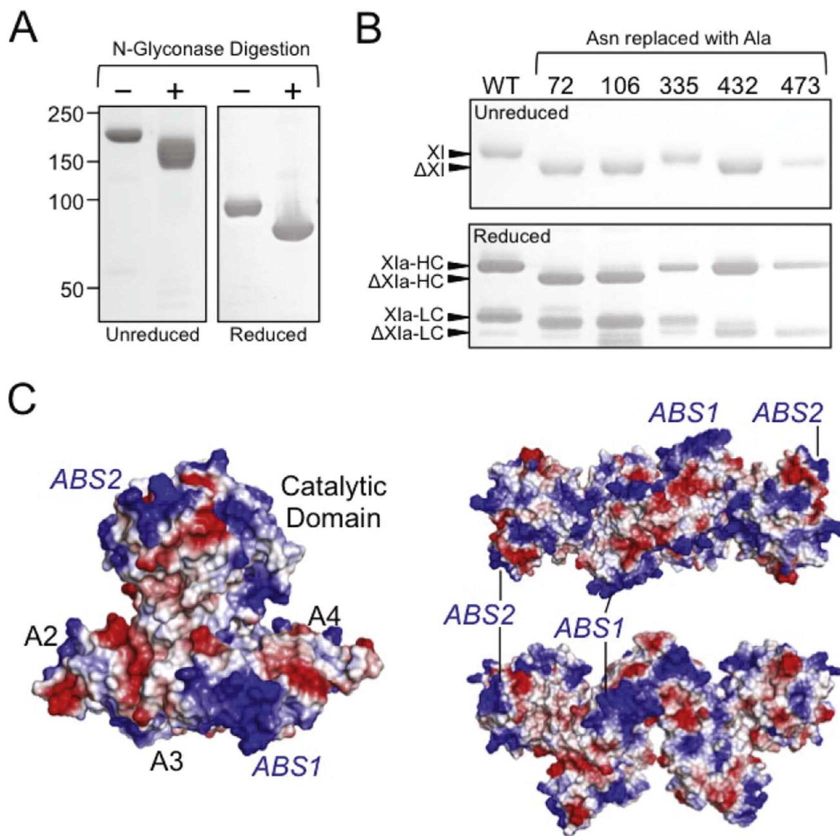


Fig. 5. Factor XI glycosylation sites and anion binding sites. (A) SDS-PAGE of human plasma derived FXI incubated in the absence (−) or presence (+) of *N*-glycosidase to remove N-linked carbohydrate moieties. Positions of molecular mass standards are shown on the left of the fig. (B) SDS-PAGE (top panel – unreduced, bottom panel – reduced) of human recombinant wild type FXI or FXIa (WT) or FXI in which specific asparagine residues (72, 106, 335, 432 or 473) have been replaced with alanine (indicated at the top). Positions of glycosylated FXI and the heavy chain (HC) and light chain (LC) of FXIa are indicated at the left of the images, as well as expected positions of FXI, HC, and LC lacking a single N-linked glycosylation site. Note that FXI with alanine replacing N335 appears similar to FXI-WT. (C) Charged surface representation of FXI zymogen monomer (left) and two views of the FXI dimer (right) showing positions of FXI anion binding sites 1 and 2 (ABS1 and ABS2). Blue indicates positively charged regions, and red negatively charged regions of the molecule. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

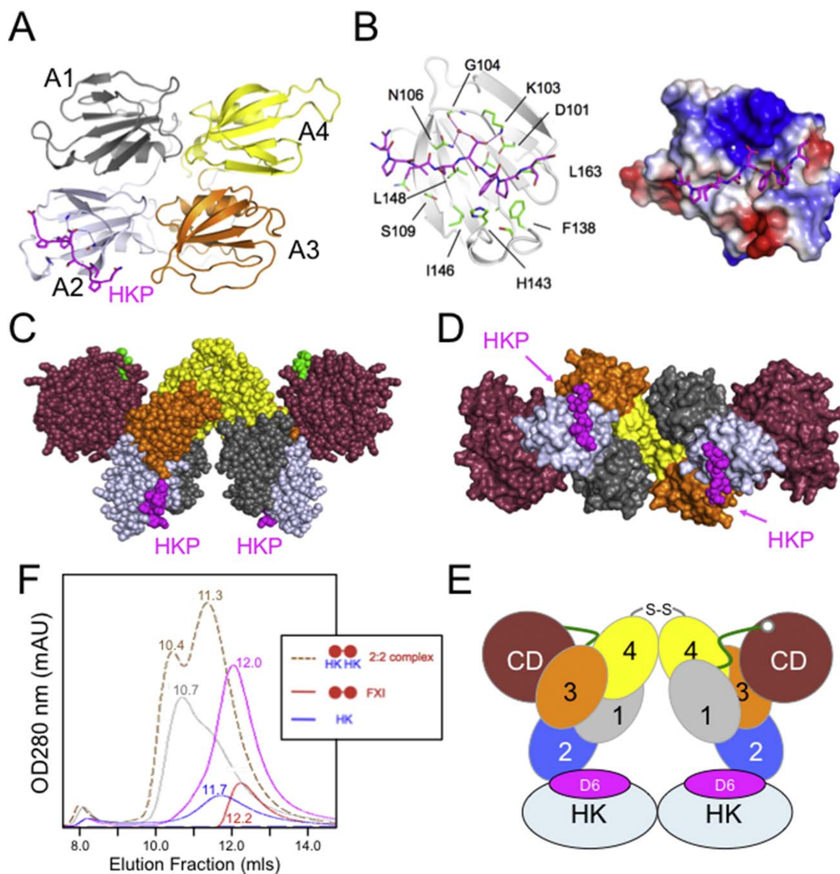


Fig. 6. The Factor XI interaction with high molecular weight kininogen. (A) Cartoon diagram of the crystal structure of the apple domain disk of FXI in complex with a synthetic peptide spanning HK amino acids 582–593 (pdb:5I25). FXI apple domains are colored grey, blue, orange and yellow for A1 to A4 domains respectively. The HK peptide (HKP-magenta) is shown as a stick figure. (B) Diagram of the FXI A2 domain (white) with the HKP peptide shown as a stick diagram in magenta. Electrostatic and hydrogen bonding interactions are shown as purple dotted lines. FXI residues interacting with HKP are shown as sticks in green. On the right is a charged surface representation of the A2 domain (blue-positive, red-negative) bound to HKP (magenta). (C) Space-filling model of FXI dimer with the catalytic domains in maroon and activation loop residues Arg^{369}—Val^{370} in green. Colors for the apple domains are as in Fig. 3. Shown is the location of FXI-bound HKP (magenta) relative to the FXI dimer axis. (D) A 90° rotated view of the FXI dimer in panel C. (E) Size-exclusion chromatography (Superdex 200) profile of UV (280 nm) absorbance *versus* retention volume (ml) for FXI (red), HK (blue), or mixtures of FXI and HK at 1:0.25 (magenta), 1:2 (grey) or 1:4 (brown-dashed) molar ratios. FXI elutes close to the expected molecular mass of the dimer (~200 kDa) and the FXI-HK complex elutes at ~400 kDa. (F) Schematic diagram of FXI interacting with HK. From Wong et al. [84]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

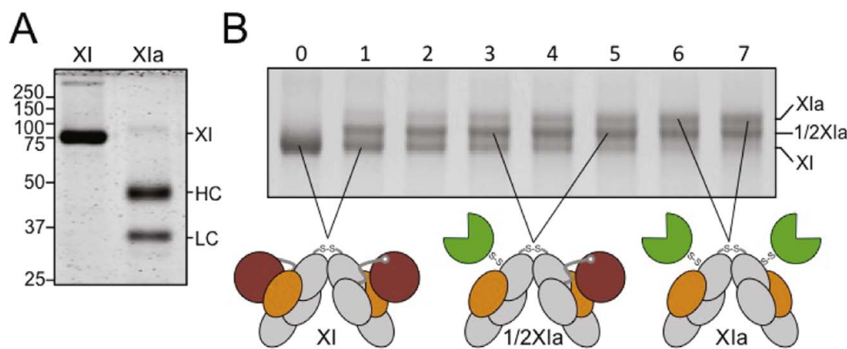


Fig. 7. Factor XI Conversion to Factor XIa. (A) Reducing SDS-PAG of FXI and FXIa showing the relative migrations of the 80 kDa FXI subunit (XI) and the heavy chain (HC) and light chain (LC) of FXIa. Positions of molecular mass standards are shown on the left in kiloDaltons. (B) Non-reducing SDS-PAGE of a time course (hours indicated at the top) of FXI activation by thrombin. Conversion of FXI to FXIa (a species with two activated subunits) proceeds through an intermediate (1/2-FXIa) with one activated subunit. The three species (FXI, 1/2-FXIa, and FXIa) migrate slightly differently on non-reducing SDS-PAGE, allowing them to be identified.

FXI in the absence of polyanions, while thrombin mediated-FXI activation is enhanced to a much greater degree by polyanions such as polyphosphate [53].

11. Factor XIa activation of factor IX

Conversion of FIX to FIXaβ is a calcium-dependent process catalyzed by factor VIIa (FVIIa) in the presence of tissue factor and phosphatidylserine-rich phospholipid [113,114], or by FXIa in a phospholipid-independent reaction [14,55,115,116]. Regardless of the activating protease, FIX is cleaved first after Arg[145] forming the intermediate FIXα, then after Arg[180] to form FIXaβ (Fig. 8A) [14,55,116]. FIXα accumulates during FIX activation by FVIIa (Fig. 8B, top), but not during activation by FXIa (Fig. 8B, bottom) [115,116]. This is not accounted for by FXIa's dimeric structure, as FIXα does not accumulate during activation by FXIa monomer [46] or 1/2-FXIa [55].

FIX binds to an exosite on the FXIa A3 domain [116–120] followed by engagement at the protease active site and cleavage of the Arg[145]–Ala[146] bond [116]. This facilitates subsequent cleavage of the Arg[180]–Val[181] bond. The catalytic efficiency for the second cleavage is 7-fold greater than for the first, explaining the minimal FIXα accumulation (Fig. 8C & D). Replacing the FXIa A3 domain with the PK A3 domain (FXIa/PKA3) reduces the catalytic efficiency for cleavage of both FIX bonds, with a greater effect on cleavage after Arg[180], leading to FIXα accumulation (Fig. 8C & D). The same "defect" is observed when FIX is activated by isolated FXIa catalytic domain (no A3 domain), or during FIX activation by FXIa in the absence of Ca^{2+} ions. Thus, FIX activation requires FIX and FIXα to bind to the FXIa A3 domain in a Ca^{2+}-dependent manner prior to catalysis.

FIX does not bind zymogen FXI [121], indicating binding sites form or are unmasked by conformational changes accompanying conversion to FXIa. Replacing Ile[183], Arg[184] and Asp[185] in the N-terminus of the A3 domain (Fig. 2) with alanine residues causes a defect in FIX activation similar to that observed with FXIa/PKA3 [117,119]. These residues form a charged ridge adjacent to a hydrophobic pocket on the A3 domain surface facing the catalytic domain, a feature not found in PK (Fig. 8E) [117]. FXI Arg[184] forms salt bridges with residues Asp[488] and Asn[566] on the protease domain (Fig. 8F), anchoring the protease domain over the putative FIX binding site (Fig. 8F) [9]. We suspect that the highly conserved Arg[184] serves as a latch that is released to expose the FIX binding site on conversion to FXIa.

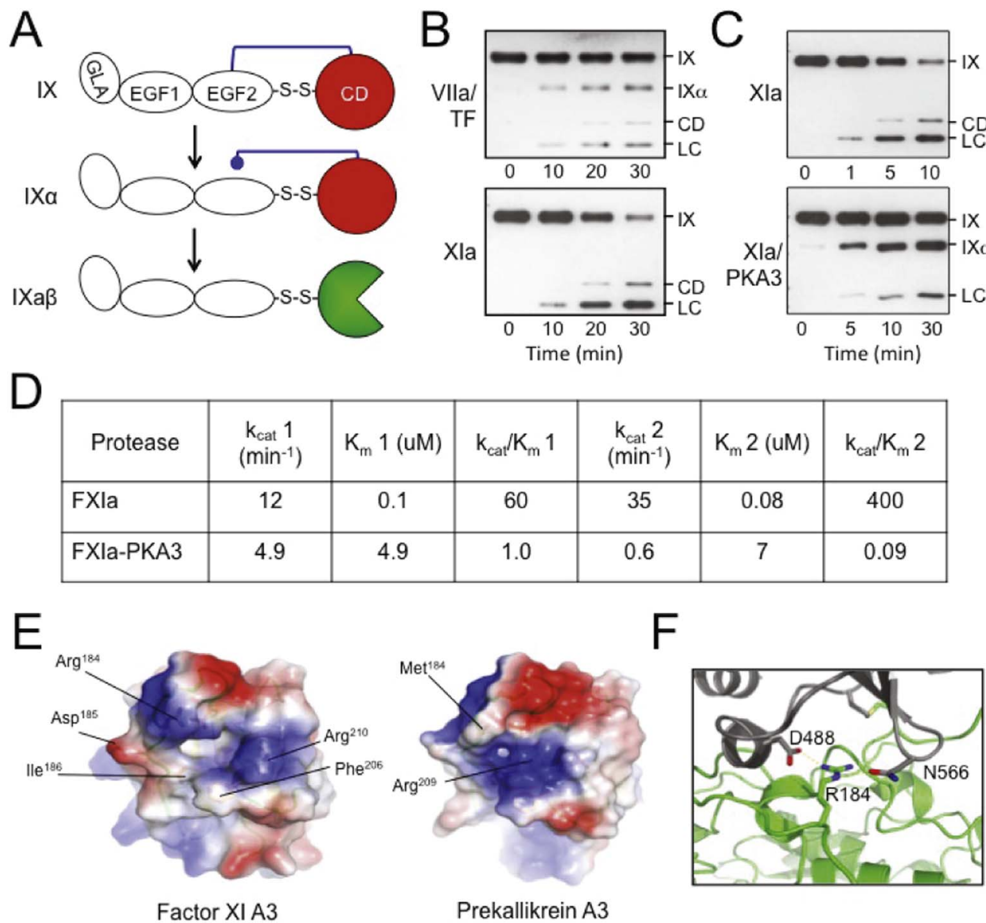
FIX binding to FXIa requires the FIX Gla domain [117,121]. Mutagenesis studies indicate that residues in the phospholipid-binding Ω-loop of the Gla-domain (residues 4 to 11) are required for FXIa binding [117] (Fig. 9A & B). The Ω-loops of vitamin K-dependent proteases mediate binding to phospholipid membranes through charged and hydrophobic interactions (Fig. 9C) [2]. The topology of the putative FIX binding site on FXIa A3 suggests an interaction involving charged and hydrophobic components. Using surface plasmon resonance, we observed that FXIa, but not FXIa/PKA3, competes with phosphatidylserine-rich surfaces for FIX binding (Messer, Bajaj, Geng and Gailani, unpublished observation), supporting the conclusion that the FIX-Gla domain engages the FXIa A3 domain. The data suggest that the A3 domain performs a role in FIX activation similar to that of phospholipid during FIX activation by FVIIa, with both interactions contributing to K_m for their respective reactions.

12. Other FXIa substrates

FXIa activates several components of the plasma thrombin generation mechanism other than FIX. Factor X (FX), a FIX homolog, is a major substrate for FIXaβ and FVIIa/tissue factor [2]. We showed that FXIa also activates FX [122]. The reaction is orders of magnitude less efficient than activation of FIX by FXIa, is not Ca^{2+}-dependent, and does not require the FXIa A3 domain. Whelihan et al. reported that FXIa cleaves factors V and VIII (FV and FVIII) to forms that have a few percent of the cofactor activity of the standard active forms FVa and FVIIIa [123]. We showed that FV activation by FXIa requires the FXIa A3 domain to proceed optimally [122], and Choi et al. reported that polyphosphate accelerates the reaction [124]. One or more of these reactions may occur *in vivo*. Mice lacking both FXI and FIX are more resistant to injury-induced thrombosis than are mice lacking only one of the proteins [122], indicating FXIa is capable of bypassing FIX to activate other clotting factors. The capacity to activate FV and/or FX may allow FXIa to influence thrombin generation by essentially jumping the gap created by FIX deficiency. The relevance of these findings to hemostasis warrants further investigation in animal models.

FXIa activates components of the KKS. Like its homolog α-kallikrein, FXIa activates FXII [18,126] and cleaves HK to release bradykinin [95,125], although neither reaction is as efficient as the α-kallikrein-mediated reactions. The kallikrein-like properties of FXIa may explain an observation made in septic mice. FXI-deficient mice have lower mortality than wild type mice with infection/inflammation induced by cecal ligation and puncture [18]. Surprisingly, FXI deficiency did not cause appreciable changes in activation of coagulation. Instead, FXI-deficient mice had a milder cytokine response, with reduced consumption of FXII and PK, indicating blunted activation of the KKS. Infusing polyphosphate into mice induces KKS activation. FXI-deficient mice receiving polyphosphate had reduced FXII and PK activation compared with wild type mice [18]. The findings support the hypothesis that FXI/XIa is not only a substrate for the KKS, but contributes to KKS activation, perhaps by activating FXII.

FXIa may promote clot formation/stability through proteolytic inactivation of regulators of coagulation. Puy et al. reported that FXIa cleaves tissue factor pathway inhibitor (TFPI) between the Kunitz 1 and 2 domains, reducing the capacity of this key protein to regulate tissue factor-initiated coagulation [127]. TFPI inhibition by FXIa is enhanced by polyphosphate chains of the length typically released by activated platelets [128]. FXIa also cleaves ADAMTS13, the von Willebrand factor-cleaving metaolproteinase, removing the C-terminal CUB



Protease	k_{cat} 1 (min^{-1})	K_m 1 (uM)	k_{cat}/K_m 1	k_{cat} 2 (min^{-1})	K_m 2 (uM)	k_{cat}/K_m 2
FXIa	12	0.1	60	35	0.08	400
FXIa-PKA3	4.9	4.9	1.0	0.6	7	0.09

Fig. 8. Factor XIa Activation of Factor IX. (A) Schematic diagrams showing conversion of factor IX to the intermediate factor IXα by cleavage after Arg[145], and then to factor IXaβ by cleavage after Arg[180]. Factor IX contains an N-terminal calcium-binding Gla-domain, two epidermal growth factor (EGF) domains, an activation peptide (blue) and a catalytic domain (CD-red). (B) Western blots of time courses of FIX activation by FVIIa/TF (top) or FXIa (bottom). Gels were run under reducing conditions. Positions of FIX zymogen (IX), the large fragment of FIXα, the light chain of FIXα and FIXaβ (LC) and the catalytic domains (CD) of FIXaβ are indicated on the right. (C) Western blots of time courses of FIX activation by FXIa (top) and FXIa with the PKA3 domain (bottom). Note the different time scales in the two panels. (D) Kinetic parameters for cleavage of FIX by FXIa or FXIa-PKA3 based on full-progress experimental traces analyzed with KinTek software (KinTek Explorer Version 2.5). K_m and k_{cat} for activation were calculated from individual rate constants for conversion of FIX to FIXα (reaction 1) and FIXα to FIXaβ (reaction 2). Catalytic efficiencies (k_{cat}/K_m) are shown for both reactions. (E) Charged surface representations of the FXI (left) and PK (right) A3 domains. Blue indicates positive charge, and red indicates negative charge. Note that the hydrophobic pocket on the surface of FXI A3 is not present in PK A3. (F) Structure of FXI showing the relationship of the A3 domain (green) and catalytic domain (grey) in the zymogen. Arg[184] and the adjacent hydrophobic pocket 1 are covered by the catalytic domain. Arg[184] forms salt bridges (dotted lines) with Asp[488] and Asn[566] in the zymogen. From [116,117]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

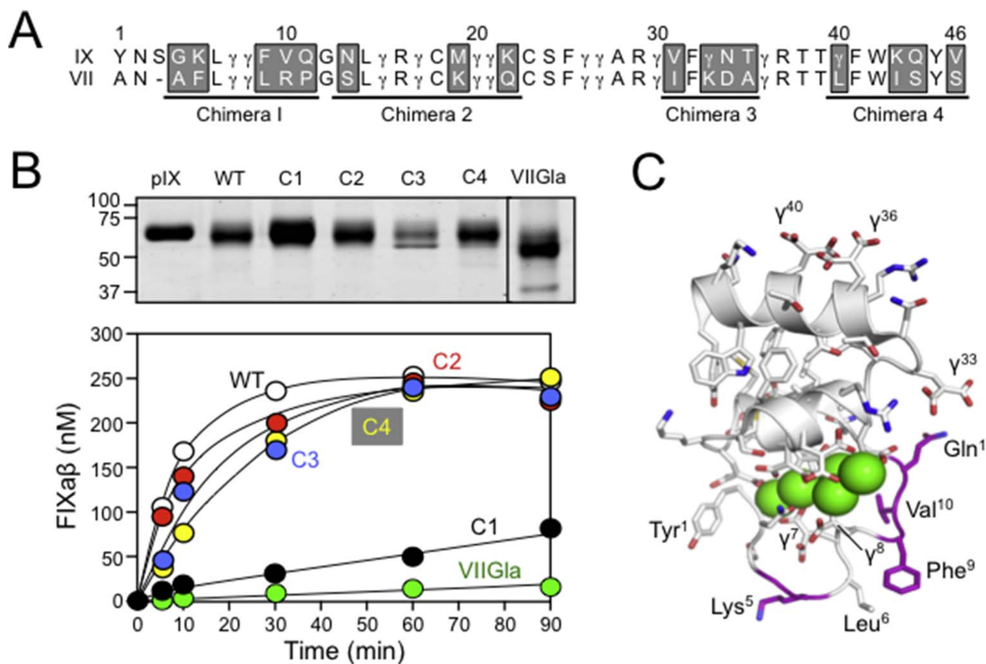


Fig. 9. Factor IX and Factor VII Gla-domain sequences. (A) Primary sequences of the human factor IX (FIX) and factor VII (FVII) Gla-domains. The numbering system shown is for FIX. The symbol γ indicates positions of γ-carboxyglutamic acid residues. Underlined sequences were changed from FIX sequence to FVII sequence to generate chimeras C1, C2, C3 and C4. The amino acids changed in each chimera are highlighted by the grey boxes. (B) Top - stained SDS-polyacrylamide gel of purified plasma FIX (pIX), recombinant wild type FIX (WT), FIX/FVII chimeras (C1, C2, C3, and C4), and FIX in which the Gla-domain from residues 1 to 46 are changed to FVII sequence (FIX/VII-Gla - abbreviated VIIGla). Bottom - FXIa (2 nM) was incubated with 250 nM FIX^{WT} (white), C1 (black), C2 (red), C3 (blue), C4 (yellow) and FIX/VII-Gla (green) (250 nM) in TBS with calcium. Shown are concentrations of FIXaβ at various times as determined by densitometry of SDS-polyacrylamide gels. (E) Topological diagram of the human FIX Gla-domain, with residues in the Ω-loop (4–11) that differ from the corresponding region of the human FVII Gla-domain highlighted (magenta). Positions of certain Gla residues are indicated by the symbol 'γ'. Calcium ions in the vicinity of the Ω-loop are represented by green spheres. The image is derived from a structure for a complex between the human FIX Gla-domain and the antibody 10C12 [142]. Figures were prepared with PYMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4). From Geng et al. [117]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

domains [129]. The result is a form of ADAMTS13 with increased activity toward small peptide substrates, but that interacts poorly with von Willebrand factor.

13. FXI interactions with platelets

FXI lacks the Gla domain that facilitates binding of vitamin K-dependent coagulation proteases to phospholipid surfaces such as membranes of activated platelets or damaged tissues [7–9]. Nevertheless, there is evidence that FXI binds to platelets, and that FXI contributes to platelet activation/accumulation in flowing blood exposed to thrombogenic surfaces. When human blood is perfused over collagen, platelets bind and aggregate in a manner supported by thrombin and fibrin formation [130–132]. Adding an anti-FXI antibody prevents fibrin formation and reduces platelet aggregate size. Anti-FXI antibodies produce a similar effect in a primate model in which thrombus-inducing collagen-coated grafts are introduced into the circulation [51,130,133].

Greengard et al. detected ~1500 FXI binding sites per activated platelet [134], with subsequent work demonstrating binding to the GP1b receptor [135]. FXI competes with von Willebrand factor, but not thrombin, for GP1bα binding [135]. Kossmann et al. recently reported that FXI localized to platelets promotes coagulation and inflammation in mice in a GP1b-dependent manner [136]. *In vitro*, optimal platelet binding involves residues in the FXI A3 domain (Fig. 2) [135,137], leucine-rich repeats at the N-terminus of GP1bα [135,138], and HK and Zn2+ ions [135,137,138]. The FXI A3 residues involved, Ser^{248}, Arg^{250}, Lys^{255}, Phe^{260} and Gln^{263} overlap with elements of ABS1 (Fig. 2). There are ~25,000 GP1b complexes per platelet, a value 10 times greater than the number of FXI binding sites, suggesting FXI binds to a subset of GP1b. White-Adams et al. showed that FXI binds to the platelet ApoER2′ receptor [139]. There are ~2000 copies of ApoER2′ per platelet, and they co-localize with GP1b. This suggests FXI binding may involve a complex of the two receptors. Preliminary data indicate the binding site for ApoER2′ on FXI is distinct from that of GP1b (McCarty and Gailani, unpublished observation).

There is evidence indicating that activated platelets support FXI activation, but the history of work in this area is complicated. A 1998 report indicating that activated platelets enhance FXI activation by thrombin at a rate 2- to 5-fold higher than dextran sulfate [80] was subsequently retracted because the results proved difficult to reproduce [140]. However, Choi et al. reported in 2011 that activated platelets and platelet releasates do enhance FXI activation by thrombin [65]. Key to this process is polyphosphate secreted by the platelets. In our experience, the effects of polyphosphate on FXI activation are inhibited in certain types of buffers, possibly explaining variation across experiments. Verhoef et al. recently reported that platelet polyphosphate chains condense into divalent cation-dependent membrane-associated particles that are considerably larger than the individual polyphosphate chains released from platelets [141]. These particles may serve as binding sites for FXI and FXII, and may be more efficient at promoting protease activation than short-chain (60–100 unit) polyphosphate species in solution.

14. Summary

FXI is structurally distinct from the vitamin K-dependent proteases that form the core of the vertebrate coagulation mechanism (Fig. 1). FXI retains structural features and activities of its parent molecule PK, and the similarities between the proteins have been invaluable in efforts to characterize unique properties of FXI. Since the duplication event that created separate PK and FXI genes, FXI has undergone important structural changes that are required for its procoagulant activities. These include adoption of a homodimeric conformation, formation of a binding site for the Gla-domain of FIX, and development of ABSs that promote activation. Changes to the activation cleavage site permitting activation by thrombin were also likely instrumental in facilitating the switch from KKS component to coagulation protease. Less clear from a pathophysiologic standpoint are the importance of FXI's conserved interaction with HK, its ability to bind to platelet receptors, and the capacity of FXIa to neutralize regulatory proteins such as TFPI and ADAMTS13. The reason FXI must be a dimer to be functional *in vivo* remains a major unanswered question. Furthermore, the major species of FXI (FXIa or 1/2-FXIa) generated during coagulation remains to be determined. Progress on these questions will be important for fully understanding the roles of FXI in coagulation and inflammation, and will better inform translational efforts to develop therapeutic strategies targeting this protein.

Acknowledgments

This work was supported by awards HL58837 and HL81326 (D.G.) and (HL130018) IMV from the National Institutes of Health, National Heart, Lung, and Blood Institute; and Programme Grant RG/12/9/29775 and Project Grant PG/09/025/27136 (J.E.) from the British Heart Foundation.

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