Thrombin Activates Factor XI on Activated Platelets in the Absence of Factor XII

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Abstract—Thrombin can activate factor XI in the presence of dextran sulfate or sulfatides. However, a physiological cofactor for thrombin activation of factor XI has not been identified. We examined this question in a cell-based, tissue factor–initiated model system. In the absence of factor XII, factor XI enhanced thrombin generation in this model. The effect on thrombin generation was reproduced by 2 to 5 pmol/L factor Xla. A specific inhibitor of factor XIIa did not diminish the effect of factor XI. Thus, factor XI can be activated in a model system that does not contain factor XIIa or nonphysiological cofactors. Preincubation of factor XI with activated platelets and thrombin or factor Xa enhanced subsequent thrombin generation in the model system. Preincubation of factor XI with thrombin or factor Xa, but without platelets, did not enhance thrombin generation, suggesting that these proteases might activate factor XI on platelet surfaces. Thrombin and factor Xa were then directly tested for their ability to activate factor XI. In the presence of dextran sulfate, thrombin or factor Xa activated factor XI. Thrombin, but not factor Xa, also cleaved detectable amounts of factor XI in the presence of activated platelets. Thus, thrombin activates enough factor XI to enhance subsequent thrombin generation in a model system. Platelet surfaces might provide the site for thrombin activation of functionally significant amounts of factor XI in vivo. (Arterioscler Thromb Vasc Biol. 1999;19:170-177.)

Key Words: factor Xla | factor XIIa | factor IXa | human blood coagulation | contact system

Coagulation factor XI circulates as a homodimer consisting of 2 identical disulfide-linked polypeptide chains (M, 80 000 each). The zymogen protein is activated by a single cleavage of each polypeptide chain to give rise to the activated form (factor Xla). Factor Xla activates zymogen factor IX to factor IXa. Unlike classic hemophilia A or B (factor VIII or factor IX deficiency), one cannot predict whether patients with factor XI deficiency will exhibit a bleeding diathesis based on their level of factor XI activity alone. Some patients who are homozygous for factor XI deficiency show a severe bleeding tendency while others are asymptomatic. Although the bleeding tendency of patients who are heterozygous for factor XI is controversial, 1 recent study showed that up to half of these patients have a bleeding tendency. Attempts have been made to correlate bleeding in factor XI–deficient patients with low levels of von Willebrand factor, blood group, and lack of an alternatively spliced form of platelet factor XI.

Activated factor XII can activate factor XI both in plasma and on activated platelets. However, deficiency of factor XII is not associated with a bleeding tendency. This led investigators to propose an alternate mechanism for activation of factor XI based on the observation that thrombin can activate factor XI in a purified system or in plasma. It remains unclear whether or not thrombin is a physiologically relevant activator of factor XI. In most studies, factor XI activation by thrombin has required the presence of dextran sulfate (DS) or nonphysiological concentrations of sulfatide cofactors. It has also been suggested that high-molecular-weight kininogen, which strongly associates with factor XI, and fibrinogen, which is the preferred substrate for thrombin, would block thrombin activation of factor XI in plasma. Another study showed that factor XI was not activated by thrombin in plasma in the presence of kaolin. However, 1 group suggested that thrombin activation of minute amounts of factor XI in plasma protected fibrin clots from fibrinolysis, presumably by increasing the amount of thrombin generated within the clot.

If thrombin is a physiological activator of factor XI, it is not known what the in vivo cofactor for this activity might be. It has been hypothesized that endogenous glycosaminoglycans or platelets might promote activation of factor XI by thrombin in vivo. One study found that a physiological glycosaminoglycan, heparan sulfate, could support activation of factor XI in plasma, but only when unphysiologically high levels of thrombin were added. Platelets can bind factor XI and provide a site for activation of factor IX by factor Xla. We hypothesized that platelets could serve as a cofactor for activation of small but physiologically important amounts of factor XI by thrombin. We have tested this hypothesis by using a defined, tissue factor–initiated, cell-based model of coagulation to examine factor XI activation by mechanisms
that are not dependent on factor XII nor on the addition of nonphysiological agents such as DS.

**Methods**

**Materials**

Macrophage serum-free medium was purchased from Life Technologies. Accu-Prep resolving media and hirudin were purchased from Accurate Chemical and Scientific Co. Chromozyme TH was purchased from Boehringer-Mannheim. TenStop was purchased from American Diagnostica. Goat polyclonal antibodies against human factor XI were purchased from Affinity Biologicals (Hamilton, Ontario, Canada). Anti-CD62 (phycoerythrin-conjugated) was purchased from Immunodymanics. All other reagents were of high commercial grade.

**Proteins**

Antithrombin III (AT III), factor IX, and prothrombin were prepared from frozen, outdated Red Cross plasma as described. C1 esterase inhibitor was purified according to a modification of previously published procedures.\(^{27,28}\) In brief, the 50% (NH\(_4\))\(_2\)SO\(_4\) precipitate was dissolved and sequentially purified on DE-52 cellulose (Whatman), SP Sepharose (Pharmacia), and Sephacryl S-200 (Pharmacia). Factors X and XIIa, c-terminal inhibitor, and single-chain, high-molecular-weight kininogen were from Enzyme Research Labs. Factors XI, Xa, and XIIa were purchased from Haematologic Technologies and factor V from Calbiochem. Factor VIII associated with von Willebrand factor (Profilelle, Alpha Therapeutics) was purchased from the hospital pharmacy at the University of North Carolina and further purified by gel filtration. Recombinant factor VIIa and recombinant, full-length, tissue factor pathway inhibitor (TFPI) were from Novo Nordisk. Prothrombin, factor IX, and factor X were treated with an inhibitor mixture (1 \(\mu\)M tosyl-Lys-chloromethyl ketone, tosyl-Phe-Ala-Arg-chloromethyl ketone, Glu-Gly-Arg-chloromethyl ketone, Phe-Pro-Arg-chloromethyl ketone, and PMSF) to inactivate proteases in the zymogens following by repurification on a Q Sepharose column (Pharmacia). Thrombin, factor IXa, and factor Xa were prepared from their zymogens as described previously. All proteins were determined to be pure on overloaded, Coomassie-stained gels.

**Cell-Based Model System**

We have described this model system in previous work.\(^{30,31}\) The components of the activated monocyte system (6 to 20 cells/\(\mu\)L of reaction mixture) cultured with lipopolysaccharide as a source of tissue factor; unactivated platelets (100 000 to 200 000/\(\mu\)L of reaction mixture); plasma concentrations of the unactivated protein factors prothrombin (1.39 \(\mu\)M; 100 \(\mu\)g/mL), factor X (135 nmol/L; 8 \(\mu\)g/mL), factor IX (70 nmol/L; 4 \(\mu\)g/mL), factor VIII (0.4 nmol/L; 1 \(\mu\)g/mL), and factor V (23 nmol/L; 7 \(\mu\)g/mL); coagulation inhibitors: TFPI (3 nmol/L; 0.1 \(\mu\)g/mL) and AT III (2.5 \(\mu\)mol/L; 150 \(\mu\)g/mL); and metal ions calcium (3 \(\mu\)mol/L) and zinc (20 \(\mu\)mol/L). In the indicated experiments factor XI at different concentrations up to its plasma level (31 nmol/L; 5 \(\mu\)g/mL) was also included. To eliminate small amounts of proteases that might have developed in the zymogens in the time since their purification, the coagulation factors and inhibitors (TFPI and AT III, and C1 esterase inhibitor when factor XI was included) were incubated together overnight in a 10\(\times\) concentrated stock solution. After incubation, the levels of active proteases were undetectable (<0.001%) by chromogenic assays (Chromozyme TH for thrombin, Spectrozyme FXa for factor Xa, and a coupled assay containing polylysine and factor X to detect factor IXa activity).\(^{29}\) A small amount of factor VIIa (0.2 nmol/L; 0.01 \(\mu\)g/mL) was added to initiate the coagulation-factors \(\times \) reactions. In some experiments, factor Xa was added to the system at the same time as the zymogens proteins and inhibitors. The protein solution was added to the cells at the start of each experiment (time=0). Samples were removed at timed intervals to assay thrombin level and extent of platelet activation.

Monocytes were isolated as previously described.\(^{32}\) In brief, blood was collected into citrate anticoagulant with 5 \(\mu\)g/mL prostaglandin E\(_1\) and separated on density gradient media (Accu-Prep). The mononuclear cell band was isolated and mononuclear cells removed from platelets by centrifugation. Remaining platelets were removed from monocytes by washing twice in cold verseine buffer. Monocytes were separated from monocytes by adherence in tissue culture plates for 1 hour at 37°C. Tissue factor expression was induced by treating monocytes for 16 to 24 hours with 500 ng/mL lipopolysaccharide.

Platelets were isolated as previously described.\(^{33}\) Platelets that had been separated from mononuclear cells as described above were gel-filtered on Sepharose CL-2B in calcium-free Tyrode's buffer (15 mmol/L HEPES, 3.3 mmol/L sodium phosphate, 138 mmol/L NaCl, 2.7 mmol/L KC1, 1 mmol/L MgCl\(_2\), and 5.5 mmol/L dextrose) with 15 \(\mu\)mol/L (1 mg/mL) BSA. Platelets were unactivated as assessed by lack of CD62 expression. Stimulation of platelets with SFLLRN-NH\(_3\) (50 \(\mu\)g/mL, 67 \(\mu\)g/mL) for 10 minutes at 37°C resulted in >95% of the population's expressing the activation marker. Platelet activation was measured, as described previously, by staining with phycoerythrin-conjugated antibody to the \(\alpha\)-granule protein CD62-P (Becton Dickinson) and analysis by flow cytometry (FACScan, Becton Dickinson).\(^{34}\)

Thrombin generation in the model system was measured by diluting samples 1:10 in assay buffer to give final concentrations of 20 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 5 mmol/L EDTA, 50 \(\mu\)mol/L TenStop (reversible inhibitor of factor Xa), and 1.0 mmol/L Chromozyme TH (thrombin chromogenic substrate). After 13 minutes, the reaction was stopped by adding an equal volume of 50% (by volume) acetic acid. Absorbance at 405 nm was measured and converted to a concentration of thrombin by comparison to a standard curve.

The high degree of sensitivity of the model system to factor Xa was used to test whether any of the coagulation proteases could activate factor XI during preincubation with activated platelets. For these experiments platelets were activated with the protease activated receptor-1 agonist peptide SFLLRN (amide form) so that no proteases were added to activate the platelets. All reactions were carried out in 3 mmol/L calcium, 20 \(\mu\)mol/L zinc. Factor X or buffer was added to the activated platelets, which were then incubated for 5 minutes at room temperature. Protease (thrombin, factor Xa, or factor IXa) or buffer was added and incubation was continued for an additional 10 minutes. A concentrated mixture of proteins containing prothrombin, factor IX, factor X, factor VIII, factor V, AT III, and TFPI was added. Factor XI was added if it was not preincubated with platelets so that all reaction mixtures contained plasma concentrations of factor XI at times of assay. Samples were removed and assayed for thrombin generation.

**Protein Labeling and Detection by Gel Electrophoresis and PhosphorImager Analysis**

Zymogen factor XI was labeled with \(^{125}\)I by the chloramine T method.\(^{35,36}\) Factor XI was labeled in phosphate buffer. Na\(^{125}\)I was purchased from Amersham and was used at 1.48 to 1.85 \(\times\) 10\(^3\) Bq (0.4 to 0.5 mCi) per 10 \(\mu\)g protein in the labeling reaction, resulting in specific activities of 0.74 to 1.85 \(\times\) 10\(^3\) Bq (2 to 5 \(\mu\)Ci) per \(\mu\)g protein. Conditions were optimized to allow for maximal preservation of clotting activity as measured by activated partial thromboplastin time (Kontact aPTT reagent, Pacific Hemostasis) with dilutions of labeled protein in factor XI-deficient plasma (HBF, Inc.). Clotting activity was measured with normal pooled plasma. Clotting activities ranged from 50% to 100% of the starting material.

\(^{125}\)I–Factor XI was repurified by Sepharose (Pharmacia) chromatography\(^1\) after labeling. Labeled protein (80 \(\mu\)g) in 0.15 mol/L NaCl, 0.1 mol/L sodium acetate, pH 5.3, with 15 \(\mu\)mol/L (1 mg/mL) BSA was bound to a 200-\(\mu\)L column. The \(^{125}\)I–Factor XI was incubated with 2 mmol/L DFP in 20 mmol/L Tris, pH 7.4, for 15 minutes on the column. The protein was then eluted with 0.5 mol/L NaCl, 20 mmol/L Tris, pH 8.1, with 15 \(\mu\)mol/L (1 mg/mL) BSA. It was incubated at pH 8.1 for \(\sim\)1 hour to allow for hydrolysis of
residual DFP\textsuperscript{37} and then gel-filtered into 0.15 mol/L NaCl, 20 mmol/L HEPES, pH 7.4, with 15 μmol/L (1 mg/mL) BSA on Bio-Rad P6 spin columns (Bio-Rad Laboratories). As reported, DFP treatment effectively inhibited factor XIIa in the zymogen factor XI, since the \textsuperscript{125}I–factor XI treated in this manner did not undergo autoactivation in the presence of DS.\textsuperscript{37} Labeled factor XI could be completely activated by factor XIa as judged by SDS–polyacrylamide gel electrophoresis and PhosphorImager analysis.

To assess factor XI activation in the presence of DS (2 μg/mL), \textsuperscript{125}I–factor XI (150 nmol/L) was incubated with 1 of the following: (1) factor XIIa (100 nmol/L) for 60 minutes at 37°C; (2) thrombin (100 nmol/L) for 30 minutes at room temperature; or (3) factor Xa (100 nmol/L, or 1 μmol/L) and hirudin (10 nmol/L) for 30 minutes at room temperature. Calcium and zinc were present in all samples. The reactions were stopped by boiling in SDS and 2-mercaptoethanol. To assess factor XI activation in the presence of activated platelets, SFLLRN-activated platelets and proteins were incubated together with (1) factor XIIa (100 nmol/L) for 60 minutes at 37°C; (2) thrombin (100 nmol/L) for 30 minutes at room temperature; or (3) factor Xa (100 nmol/L, or 1 μmol/L) and 2-mercaptoethanol. To assess factor XI activation in the presence of DS and 2-mercaptoethanol. Samples were electrophoresed on 10% to 15% gradient gels as previously described using the PhastSystem (Pharmacia).\textsuperscript{30} Gels were fixed for 15 minutes in 50% methanol, 10% acetic acid, followed by a brief wash in 5% glycerol, 7.5% acetic acid. The fixed gels were exposed to PhosphorImager screens scanned at 50 μm per pixel resolution on a STORM 860 unit and analyzed with ImageQuant software (Molecular Dynamics). Unlike simple autoradiography with exposure of gels to x-ray film, the PhosphorImager has a linear response to the level of radioactivity over a broad range of counts per minute. It can, therefore, be used to obtain more reliable quantitative data than can exposure of the gels to film followed by densiometric scanning. Use of ImageQuant software allowed us to determine the relative amount of radioactivity in factor XI zymogen and factor XIa heavy- and light-chain bands. The “percent conversion” of factor XI to XIa is the total amount of radioactivity in the heavy- and light-chain regions divided by the sum of the radioactivity in the factor XI and XIa bands. The background level of conversion of factor XI to XIa was 1% to 2% when factor XI was incubated with (1) DS alone (no protease); (2) thrombin alone (no DS or platelets); (3) factor Xa alone (no DS or platelets); or (4) platelets alone (no DS or protease).

**Results**

We have developed a model system that contains a cellular source of tissue factor; unactivated platelets; and plasma concentrations of zymogen coagulation factors and coagulation inhibitors (detailed in Methods). In the current study, we used this model to examine the activation and activity of factor XI under conditions that mimic in vivo coagulation. Zymogen factors (IX, X, VIII, V, and prothrombin), calcium, AT III, TFPI, and a catalytic amount of factor VIIa are added to unactivated platelets and a cellular source of tissue factor. Platelet activation and thrombin generation were measured at timed intervals. Addition of factor XI to the reaction mixtures enhanced thrombin generation in assays using platelets from 12 different donors (results from 9 donors are shown in Figure 1). We have previously shown that platelets from different individuals support different levels of thrombin generation when incubated with identical coagulation protein mixtures.\textsuperscript{38} Although the lag phase before the onset of detectable thrombin generation and the degree of enhancement varied between donors, thrombin generation was increased in every case in the presence of factor XI.

Representative results from 1 donor are shown in Figure 2, although the pattern was the same when platelets from 2 additional donors were used. Addition of factor XI increased thrombin generation in a dose-dependent manner (panel B). The effect of factor XI on thrombin generation was not due to enhanced platelet activation, because the time required for platelet activation (3 to 4 minutes) was not altered (panel A). As little as 5% of normal plasma levels of factor XI (100% = 5 μg/mL, 31 nmol/L) gave some increase in thrombin generation, with a maximal effect at 50% of normal. The effect of factor XI was dependent on the presence of both factors IX
zymogen factor XI on thrombin generation could be due to factor XIIa in the presence of DS.

We used was active, because it enhanced factor XI activation. Others have reported that factor XI activation by thrombin could be detected after this treatment. To enhance thrombin generation in the model system, small amounts of factor XIa were added instead of plasma levels of zymogen factor XI. Factors IX, X, VIII, V, and prothrombin, metal ions, AT III, and TFPI were incubated with tissue factor–bearing monocytes and unactivated platelets. Concentrations of factor XIa included in the reactions were none (open squares), 0.1 pmol/L (filled circles), 1 pmol/L (filled squares), or 10 pmol/L (open circles). Amount of thrombin is plotted against sampling time.

High-molecular-weight kininogen at its plasma level (80 μg/mL) had no significant effect on the rate of thrombin generation. Factor XIIa did accelerate thrombin generation at a final concentration of 7 μmol/L (a concentration above that which completely inhibited factor XIIa in a chromogenic assay) did not decrease the amount of thrombin generated in the presence of factor XI. Thus, although the model system is quite responsive to the level of factor XI(a), we found it to be insensitive to factor XII and high-molecular-weight kininogen. Addition of plasma concentrations of factor XII (30 μg/mL, 37.5 nmol/L) or up to 100 pmol/L factor XIIa (either with or without factor XI and high-molecular-weight kininogen) had no significant effect on the rate of thrombin generation. Factor XIIa did accelerate thrombin generation when added at 1 nmol/L or greater, but only when factor XI was also present.

If factor XI is activated in a model system that lacks factor XII, then activation must be mediated by a protease(s) generated in situ. Therefore, we tested the ability of thrombin, factor Xa, and factor IXa to activate factor XI on the surface of activated platelets. In these assays, tissue factor–bearing cells were omitted. Instead, platelets were activated with the thrombin receptor tethered-ligand peptide SFLLRN-NH₂ as described in “Methods.” Factor XI (or buffer for control experiments) was then incubated with activated platelets for 5 minutes. Either buffer or 100 pmol/L thrombin, factor Xa, or factor IXa was then added and incubated for an additional 10 minutes. Because addition of even very small amounts of factor XIa will increase thrombin generation in the cell-based model system (Figure 3), we used a modification of this system to detect the small amounts of factor XIa formed during preincubation of factor XI with platelets and a protease. The dilemma in designing these experiments is this: addition of greater amounts of protease, which might lead to greater amounts of factor XI activation, also increases background thrombin generation, which could obscure any effect on factor XI activation. The concentrations of proteases were chosen so that thrombin generation due to addition of contamination with trace amounts of factor XII or factor XIIa. To do this, we used a very specific inhibitor of factor XIIa activity, corn trypsin inhibitor. To provide factor XII(a) with the best chance of exerting an effect, we also included high-molecular-weight kininogen at its plasma concentration in the assay system. Corn trypsin inhibitor at a final concentration of 7 μmol/L (a concentration above that which completely inhibited factor XIIa in a chromogenic assay) did not decrease the amount of thrombin generated in the presence of factor XI. Thus, although the model system is quite responsive to the level of factor XI(a), we found it to be insensitive to factor XII and high-molecular-weight kininogen. Addition of plasma concentrations of factor XII (30 μg/mL, 37.5 nmol/L) or up to 100 pmol/L factor XIIa (either with or without factor XI and high-molecular-weight kininogen) had no significant effect on the rate of thrombin generation. Factor XIIa did accelerate thrombin generation when added at 1 nmol/L or greater, but only when factor XI was also present.

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protease alone was as small as possible. After the above preincubation steps, a concentrated mixture of zymogen proteins and inhibitors was added, and thrombin generation was measured. As shown in Figure 5, when factor XI was preincubated with platelets without added protease, little thrombin generation occurred. This result shows that there was no significant contamination of zymogen factor XI with factor XIa. It also confirms that factor XI was not being activated by a platelet protease or platelet-associated factor XII(a). Preincubation of platelets and factor XI with thrombin led to an increase in the amount of thrombin subsequently made on the platelet surface. Surprisingly, preincubation of factor XI with platelets and factor Xa also led to an increase in thrombin generation. Preincubation with platelets and factor IXa had no effect on thrombin generation occurred. This result shows that there was no significant contamination of zymogen factor XI with factor Xa. It also confirms that factor XI was not being activated by a platelet protease or platelet-associated factor XII(a). Preincubation of platelets and factor XI with thrombin led to an increase in the amount of thrombin subsequently made on the platelet surface. Surprisingly, preincubation of factor XI with platelets and factor Xa also led to an increase in thrombin generation. Preincubation with platelets and factor IXa had no effect on thrombin generation (data not shown). The use of platelets from different donors enhanced thrombin generation to different degrees after preincubation with factor XI and thrombin or factor Xa. It also confirms that factor XI was not being activated by a platelet protease or platelet-associated factor XII(a). Preincubation of platelets and factor XI with thrombin led to an increase in the amount of thrombin subsequently made on the platelet surface. Surprisingly, preincubation of factor XI with platelets and factor Xa also led to an increase in thrombin generation. Preincubation with platelets and factor IXa had no effect on thrombin generation (data not shown). The use of platelets from different donors enhanced thrombin generation to different degrees after preincubation with factor XI and thrombin or factor Xa. Preincubation of factor XI with thrombin or factor Xa in the absence of platelets had no effect on subsequent thrombin generation (data not shown). These results demonstrate an enhancement of thrombin generation that is due to preincubation of zymogen factor XI with thrombin or factor Xa in the presence of activated platelets and suggest that under these conditions, factor XI is being activated.

The amount of factor XIa generated under conditions in the model system was too small to be detected directly or visualized by gel electrophoresis. To directly examine the ability of coagulation proteases to cleave factor XI, we incubated high levels of thrombin and factor Xa with factor XI in the presence of DS or activated platelets. As a control, factor XI was also incubated with factor XIIa. Although incubations with factor Xa and thrombin were done for 30 minutes at room temperature, incubations with factor XIIa...
were done for 1 hour at 37°C to ensure that clearly visible heavy- and light-chain bands were present. As shown in the upper portion of Figure 6, our data are in agreement with previous reports that thrombin cleaves 125I–factor XI in the presence of DS. Incubation of factor XI with thrombin yielded bands with the same mobility as those produced by cleavage with factor XIIa. In a novel observation, we also note activation of factor XI by factor Xa in the presence of DS. Factor Xa was, however, less efficient than thrombin in activating factor XI. PhosphorImager analysis of the gels revealed that under our incubation conditions, factor XIIa converted \( \approx 40\% \) of the 125I–factor XI to factor Xla, whereas thrombin converted \( \approx 15\% \) and factor Xa, \( \approx 4\% \). Factor IXa did not activate factor XI above background levels in the presence of DS (data not shown). No evidence of autoactivation of factor XI in the presence of DS was detected, indicating that trace contamination of the zymogen with factor XIa was not responsible for the cleavage observed in the presence of added proteases. Also, incubation of factor XI with proteases in the absence of DS resulted in no cleavage above background (data not shown). Addition of plasma concentrations of high-molecular-weight kininogen reduced cleavage of factor XI by factor XIIa 44,45 did not reduce the effect of factor XI in the model system, but had little effect on the rate of platelet activation. Thus, in the absence of factor XII, factor XI did not play a role in initiating “coagulation” in this model but instead participated in a positive-feedback loop that enhanced platelet-surface thrombin generation. We conclude that the effects of factor XI in the model system were due to its activation to factor Xla for 2 reasons. First, addition of factor XI did not lead to thrombin generation unless factor IX and factor X were also present. Second, addition of \( \approx 1 \) to 10 pmol/L factor Xla reproduced the effects of plasma concentrations of the zymogen (31 nmol/L).

Our results suggest that only a small proportion of the total factor XI is activated during the process of “coagulation” in the model system. Because 2 to 5 pmol/L factor Xla added to the fluid phase reproduces the effects of zymogen factor XI in the model system, it suggests that much less factor XI is actually being activated on the platelet surface. If no more than 2 to 5 pmol/L factor Xla were produced, this amount would represent \( < 0.02\% \) conversion of the zymogen to active protease. This is consistent with the results of von dem Borne et al.,21 who reported that thrombin-dependent activation of factor XI, as measured by inhibition of fibrinolysis by thrombin activatable fibrinolysis inhibitor, represented \( \approx 0.01\% \) conversion of zymogen factor XI to factor Xla.

Conversion of trace amounts of factor XI to Xla, well below the limits of detection by autoradiography, can completely account for the effects of plasma levels of factor XI in our model system. Therefore, it is futile to try to monitor the activation of factor XI by direct physical means, such as gel electrophoresis. A highly amplified functional system appears to be necessary to detect the tiny amounts of factor Xla responsible for physiological activity.

Because the experimental system we used is so sensitive, the possibility had to be rigorously excluded that small amounts of activated factors were contaminants in our protein preparations. Therefore, all zymogen factors were subjected to prolonged incubation with inhibitors to eliminate trace amounts of activated coagulation factors before being used in experiments.30 Incubation of factor XI and coagulation factors treated in this manner with activated platelets, but without any additional initiator, did not lead to thrombin generation. Therefore, there was no significant contamination of the zymogen proteins with factor Xla.

Even though neither factor XII nor Xlla was included in the reaction mixture, it was possible that tiny amounts could be present as contaminants in the purified proteins, especially in factor XI and high-molecular-weight kininogen. Additionally, it is possible that traces of factor XII could be released from platelet \( \alpha \)-granules, which contain a variety of procoagulant proteins including factor VIII/von Willebrand factor,40 factor IX,41 factor V,42 and high-molecular-weight kininogen.43 We confirmed that the small amounts of factor Xla generated in the model system did not result from contaminating factor Xlla by using a specific inhibitor of factor Xlla. Corn trypsin inhibitor49 at a concentration between 300- and 3000-fold above its \( K_i \) for inhibition of factor Xlla44,45 did not reduce the effect of factor XI in the model system. The ability of factor XI to enhance thrombin generation was also not affected by high-molecular-weight kinino-

Discussion

We have examined the effect of factor XI in a cell-based model of coagulation that has been previously shown to mimic aspects of in vivo coagulation.30,31 This system does not include added factor XII or factor Xlla and can therefore be used to study factor XII–independent activation of factor XI. The coagulation reactions in our model system are initiated by a small amount of added factor VIIa acting in concert with cell-associated tissue factor. Only protein and cellular components that would be present in vivo are included. Inclusion of plasma concentrations of zymogen factor XI markedly enhanced the generation of thrombin in this model system but had little effect on the rate of platelet activation.
gen. We found that our model system was generally insensitive to the effects of factor XII. This is, of course, different from the case with plasma clotting assays wherein factor XII is an excellent initiator of coagulation. We do not know whether the insensitivity of the model system to factor XII and to high-molecular-weight kininogen reflects the situation in vivo or reveals a shortcoming of the model. However, it is generally recognized that deficiencies of factor XII and high-molecular-weight kininogen do not lead to a clinical bleeding diathesis, and thus, these factors may not play a role in normal hemostasis.

We have shown that both thrombin and factor Xa, but not factor Xa, appear to activate factor XI when a catalytic amount of the protease is preincubated with zymogen factor XI and activated platelets. The preincubation protocol allows zymogen factor XI to first bind to the platelet surface and then for the protease to activate it. Activation of factor XI is detected as enhanced thrombin generation compared with control (background) samples. This amplification technique suggests that factor Xa might play a role in activating factor XI. However, in these experiments we cannot rule out the possibility that added factor Xa binds to factor V(a) released from activated platelets and activates small amounts of prothrombin released from platelets. Thus, thrombin generated during preincubation could be responsible for activating factor XI.

We hypothesized that platelets might provide a surface that promotes activation of factor XI by thrombin. In experiments to directly detect activation of factor XI by coagulation proteases, we found that both thrombin and factor Xa can activate factor XI in the presence of DS. It has not been reported previously that factor Xa can serve as an activator of factor XI on any surface. When activated platelets were added to provide a surface for factor XI activation, thrombin consistently activated small amounts of factor XI; factor Xa, however, did not.

A number of factors might influence factor XI activation in vivo. Fibrinogen has been reported to reduce factor XI activation by thrombin,20 because it is a more abundant and desirable substrate for thrombin in plasma. The model coagulation system used in this study has thrombin generation as an end point, and we have not evaluated the effects of fibrinogen.

High-molecular-weight kininogen has also been reported to inhibit thrombin activation of factor XI.16,17 In our experiments to visualize cleavage of factor XI by thrombin and factor Xa, addition of high-molecular-weight kininogen reduced the amount of factor XI activated by thrombin on platelets. Baglia and Walsh46 have shown that high-molecular-weight kininogen and thrombin compete for binding to the first alpha domain of factor XI. These authors have further demonstrated that prothrombin abolishes the ability of high-molecular-weight kininogen to inhibit thrombin-mediated factor XI activation.23 Consistent with their results, addition of high-molecular-weight kininogen to the model system did not reduce thrombin generation in the presence of factor XI. We speculate that prothrombin prevented factor XI from binding to high-molecular-weight kininogen and allowed activation by thrombin and/or factor Xa.

We have shown that thrombin is capable of activating functionally significant amounts of factor XI in the presence of activated platelets without the addition of any nonphysiological cofactor. We have also shown that factor Xa can directly cleave factor XI under some circumstances. Our studies do not necessarily exclude a role for factor XIa in activation of factor XI in vivo. However, they do suggest that factor XI could be activated by thrombin and/or factor Xa in vivo on the surface of activated platelets in amounts sufficient to enhance coagulation.

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References


Thrombin Activates Factor XI on Activated Platelets in the Absence of Factor XII
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