Factor XI Antigen and Activity in Human Platelets

By George P. Tuszynski, Sandra J. Bevacqua, Alvin H. Schmaier, Robert W. Colman, and Peter N. Walsh

Washed platelets, contaminated with less than 0.20% plasma factor XI, were examined for the presence of factor XI antigen and activity. These platelets contained a factor-XI-like coagulant activity (0.67 ± 0.11 U/10^11 platelets) that remained constant after successive washes. By means of indirect immunofluorescence, a monospecific antibody to factor X1 showed specific staining of both normal platelets and platelets from patients deficient in plasma factor XI. Radiolabeled Triton extracts of washed platelets and labeled purified factor XI solutions were analyzed by SDS gel electrophoresis. On unreduced gels, the platelet material ran as a single band having an apparent molecular weight of 220,000 daltons, whereas purified plasma factor XI gave a single band at 160,000 daltons. On reduced gels, the platelet material analyzed as a single band at 52,000 daltons, whereas purified factor XI gave a single band of 80,000 daltons. Analysis of a partially purified factor XI preparation from platelets by immunoelectrophoresis revealed that the platelet preparation displayed a slightly lower cathodal electrophoretic mobility at pH 8.6 than did plasma factor XI and yet appeared to possess complete antigenic identity with plasma factor XI. These results indicate that platelets possess a form of factor XI that exists as a disulfide-linked 52,000-dalton tetramer in contrast to the plasma form that circulates as a 80,000-dalton disulfide-linked dimer.

HUMAN PLATELETS can participate in the interaction of intrinsic coagulation proteins, including those of the “contact phase,” i.e., factor XII, prekallikrein, high molecular weight kininogen, and factor XI. Activated platelets may provide a surface that sequesters these proteins and promotes the proteolytic activation of factors XII and XI. Additional evidence indicates that platelets can promote a kallikrein-dependent proteolytic activation of plasma factor XI in the absence of added factor XII. It is important, therefore, to identify and characterize any intrinsic (i.e., platelet-associated) coagulation proteins that might influence these and subsequent coagulation reactions.

Evidence for a “surface factor” in platelets has been variously interpreted as factor XIIa, factor XI, or factor-XI-like activity. Although it is presently agreed that the contact factor coagulant activity in platelets mimics that of the zymogen form of factor XI, attempts to demonstrate factor XI antigen in platelets have been unsuccessful. Studies from our laboratory have shown that the factor XI activity resides in the plasma membrane fraction, is not inactivated by anti-factor-XI antibody, and is present in platelets from a hemostatically normal patient with congenital absence of plasma factor XI. The present studies were carried out to explore the nature of the intrinsic factor XI activity of platelets. We present immunologic evidence that well washed and characterized platelet suspensions contain factor XI antigen, identified by three independent techniques: immunofluorescence, immunoelectrophoresis, and immunoprecipitation.

MATERIALS AND METHODS

Reagents

Proteolytic enzyme inhibitors, buffers, common reagents, and reagents for clotting assays were purchased from Sigma Chemical Co., St. Louis, Mo. Barbital buffer mix B-2, pH 8.6, from Harleco suitable for immunoelectrophoresis was purchased from Educational Products, McGraw Park, Ill. Agarose highest electroendosmotic grade was obtained from Marine Colloids, Inc., Rockland, Me. Chromatographic resins were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Reagents for sodium dodecyl sulfate (SDS) gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. FITC-labeled goat anti-rabbit IgG was purchased from Miles Laboratories, Inc., Elkart, Ind. Ten percent suspensions of fixed Staphylococcus aureus Cowan I were kindly supplied by Dr. E. Frank of the Wistar Institute, University of Pennsylvania, Philadelphia. Microscope slides, no. 00800, with small basins on a black background, especially designed for immunofluorescence studies of suspension cultures, were obtained from Carlson Scientific Inc., Peotone, Ill. Human albumin antiserum was purchased from Cappell Laboratories, Downingtown, Pa.

Assays

Protein assays were performed according to the procedure of Lowry et al. Factor XI clotting assays were performed as previously described.
Isolation of Platelets

Platelets were isolated by three washing procedures. For the immunofluorescence and clotting studies, platelets were washed three times by the albumin density gradient procedure, followed by gel filtration in HEPES buffered Tyrode solution, pH 7.3. Platelets used in the preparation of platelet extracts were isolated from plasma by centrifugation followed by gel filtration (see "Partial Purification of Platelet Factor XI"). In experiments designed to assess contamination of platelet suspensions with plasma proteins, 125I-labeled bovine serum albumin or 131I-labeled factor XI were added to platelet concentrates before washing by either procedure. Platelets that were used for direct immunoprecipitation analysis were washed by the method of Mustard et al.

Purification of Factor XI High Molecular Weight (HMW) Kininogen and Anti-Factor-XI Antibody

Purified factor XI and anti-factor-XI were prepared as described elsewhere. Immunodiffusion analysis of the purified factor XI antibody revealed one line against purified factor XI (Fig. 1). No lines against IgG, platelet extract, or plasma samples could be detected. Similar results were observed when immunoelectrophoretic analysis was applied to these samples (data not shown). Apparently, factor XI antigen levels in plasma and platelet extracts are too low to be detected by these methods without some prior factor XI concentration step, such as the Con-A procedure described below. Factor XI preparations were greater than 95% pure, giving specific activities of 230 U/mg protein, contained no immunoglobulin. The antibody was specific for factor XI as assessed by clotting assays. HMW kininogen was purified by the method of Kerbiriou et al., giving 0.26 x 10^6 cpm/mg protein. Dialyzed platelet extract used in the labeling of platelet extracts and purified proteins was radiolabeled with 125I by the method of McFarlane and free iodine removed by the method of Turowski et al. Purified factor XI retained full coagulant activity after radiolabeling and contained 2.06 x 10^6 cpm/μg protein. Dialyzed platelet extract used in the direct immunoprecipitation analysis was labeled with 125I by the chloramine-T procedure. The extract incorporated 400 mCi of label when 1 mCi of 125I was used.

Indirect Immunofluorescence

Indirect immunofluorescence was performed by the modified procedure of von dem Borne et al. Briefly, 5 μl of a platelet suspension (50,000/μl) was spotted onto each sample basin of a microscope slide. The slides were air dried and dipped into a solution of 1% paraformaldehyde in 15 mM phosphate buffer, containing 0.15 M NaCl (PBS), for 5 min. The fixed platelets on the slides were washed 3 times in PBS, and the platelets were permeabilized by dipping for 30 sec into acetone cooled to -20°C. To the dried platelet samples, 5 ml of various dilutions of the first antibody were applied and incubated in a humidor (a covered Petri dish containing wet filter paper) for 3 hr at 37°C. The slides were washed 3 times with PBS, excess moisture removed with filter paper leaving the sample moist, and various dilutions of the FITC-goat anti-rabbit IgG applied and incubated in a humidor for 3 hr at 37°C. At the end of the incubation, the slide was washed 3 times in PBS and once in water, excess moisture removed, and mounted with 50% glycerol in PBS. The slides were then viewed immediately under fluorescence microscopy at 200 times magnification, with an Olympus microscope equipped to view fluorescein fluorescence.

Partial Purification of Platelet Factor XI

Platelet extracts were prepared from Red Cross platelet concentrates. Platelet-rich plasma (PRP) was incubated for 30 min at 37°C with benzamidine (10 mM), epsilon amino caproic acid (EACA, 5 mM), EDTA (2 mM), soybean trypsin inhibitor (SBTI, 0.02%), hirudin (0.2 units/ml), and aprotonin (50 KIU/ml). After incubation, the platelet suspension was centrifuged for 15 min at 800 g at 23°C. The platelet pellets were resuspended in a minimal volume (usually 1/25 to 1/30 of the original PRP volume) of calcium-free Tyrode's solution, buffered with HEPES, pH 7.35, containing benzamidine (10 mM), EACA (5 mM), EDTA (2 mM), SBTI (0.02%), aprotonin (50 KIU/ml), rotenone (20 mM), and 2-deoxy-d-glucose (5 mM). The resuspended platelets were incubated with these inhibitors for 20 min at 37°C and then gel filtered (4 ml of platelet suspension per column) on 2.8 x 9.8 cm columns of Sepharose 2B in HEPES-buffered Tyrode's solution. The void volume fractions were pooled and pelleted by centrifugation at 800 g at 23°C. This second platelet pellet was resuspended in one-tenth volume of material prior to gel-filtration in HEPES-buffered Tyrode's solution containing N-CBZ-glycyl L-phenylalanine (15 mM) and phenylmethyl sulfonylfluoride (PMSF) (2 mM). The sample was then placed on ice and 2-mercaptoethanol (2-ME) and diisopropyl fluorophosphate (DFP) were added to give final concentrations of 5 mM. After incubating 5 min at 37°C, the platelets were solubilized at 4°C with 0.2% Triton X-100. The solubilized platelet columns were centrifuged at 14,000 g for 5 min. The fixed platelets on the slides were washed 3 times in PBS, and the platelets were permeabilized by dipping for 30 sec into acetone cooled to -20°C. To the dried platelet samples, 5 ml of various dilutions of the first antibody were applied and incubated in a humidor (a covered Petri dish containing wet filter paper) for 3 hr at 37°C. The slides were washed 3 times with PBS, excess moisture removed with filter paper leaving the sample moist, and various dilutions of the FITC-goat anti-rabbit IgG applied and incubated in a humidor for 3 hr at 37°C. At the end of the incubation, the slide was washed 3 times in PBS and once in water, excess moisture removed, and mounted with 50% glycerol in PBS. The slides were then viewed immediately under fluorescence microscopy at 200 times magnification, with an Olympus microscope equipped to view fluorescein fluorescence.

Labeling of Platelet Extracts and Purified Proteins

Platelet extracts, purified factor XI, and bovine serum albumin were radiolabeled with 125I by the method of McFarlane and free iodine removed by the method of Turowski et al. Purified factor XI retained full coagulant activity after radiolabeling and contained 2.06 x 10^6 cpm/μg protein. Dialyzed platelet extract used in the direct immunoprecipitation analysis was labeled with 125I by the chloramine-T procedure. The extract incorporated 400 mCi of label when 1 mCi of 125I was used.

Fig. 1. Immunodiffusion of anti-factor-XI antibody. Sample wells were loaded with 8 μl of solution. The concentration of purified factor XI was 0.32 mg/ml, the concentration of human IgG was 10 mg/ml, and the concentration of a 0.5% Triton extract of platelets was 20 mg/ml.
**Immunoelectrophoresis**

Immunoelectrophoresis was performed on platelets and platelet extracts using 1% agarose in B-2 buffer, pH 8.6, for 2–3 hr at 15°C at 250 V. Plates were stained for protein with Coomassie blue. Radial immunodiffusion was performed using B-2 buffer according to the procedure of Mancini et al.

**Gel Electrophoresis**

SDS-gel electrophoresis was performed according to the procedure of Laemmli.

**Immunoprecipitation**

Immunoprecipitation analysis of the unfractionated platelet extract was performed essentially by the procedure of Kesseler. All incubation mixtures contained 0.5% Triton or 0.5% NP-40. These nonionic detergents do not interfere with antibody antigen reactions. Platelet extracts that were used for direct immunoprecipitation analysis were washed as described above, and 3 x 10^6 platelets were suspended in 0.5 ml of HEPES-buffered Tyrode’s, pH 7.5, containing 1 mg/ml bovine serum albumin (BSA). The suspension was made 1 mM in DFP before addition of Triton to a final concentration of 0.5%. All undissolved material was centrifuged at 10,000 g for 2 min, and 100 μl of the supernatant dialyzed against 0.1 M borate buffer, pH 8.6, overnight in the cold. The dialyzed solution was then radiolabeled with 125I as described above.

Incubation mixtures consisted of 5 μl (20 μCi) of labeled Triton extract and 10 μl of the antiserum to be tested. After 1-hr incubation at 37°C, 100 μl of a 10% suspension of washed Staph was added to each tube and the mixture incubated on ice for 10 min. The adsorbed Staph was then washed 4 times by centrifugation in microcentrifuge for 2 min using NET buffer containing 0.05% NP-40. The washed Staph pellet was extracted with 200 μl Laemmli SDS sample buffer containing 4 M urea by boiling, for 2 min. After boiling, the samples were spun in a microcentrifuge for 2 min and the supernatants were electrophoresed untreated or with 5% β-mercaptoethanol added.

**RESULTS**

**Contamination of Platelet Suspension With Plasma Factor XI**

To address the possibility that our washed platelet suspensions were contaminated with plasma factor XI, several control experiments were performed. Albumin density washed platelets were analyzed for factor XI clotting activity after successive washes and found to contain unchanging levels of factor XI activity after two washes (Fig. 2). The amount of factor XI activity in washed platelet suspensions was 0.67 ± 0.11 SEM U/10^11 platelets (mean of 22 measurements).

In order to measure the amount of plasma factor XI retained in our washed platelet suspensions, the following experiments were performed. To 100 ml of a platelet concentrate containing 1.67 x 10^11 platelets and 101 U of plasma factor XI coagulant activity was added 0.16 μg of purified 125I-labeled plasma factor XI (total radioactivity equals 3.3 x 10^6 cpm, see Table 1). After centrifugation and gel filtration, the amount of 125I-labeled factor XI remaining equaled 0.19% of that initially added or 0.20 U, whereas the total factor XI coagulant activity measured was 2.10 U. This means the washed platelets contained at least tenfold more factor XI coagulant activity than could be accounted for by contamination from plasma factor XI. Stated in another way, at least 90% of the factor XI activity of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Labeled Factor XI (cpm)</th>
<th>Total Factor XI Activity (U)</th>
<th>Total Plasma Factor XI (U)</th>
<th>Percent Plasma Factor XI Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma washed</td>
<td>331,743</td>
<td>101</td>
<td>101</td>
<td>9.5†</td>
</tr>
<tr>
<td>platelets</td>
<td>640</td>
<td>2.10</td>
<td>0.20*</td>
<td>9.5†</td>
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One-hundred milliliters of platelet concentrate containing 1.67 x 10^11 platelets/μl and 0.158 μg of labeled plasma factor XI (331,743 cpm) were washed according to the procedure described in Methods. Total factor XI radioactivity and factor XI clotting activity of the washed platelet suspensions were measured.

*Calculated from fraction of labeled factor XI remaining times 101 units.
†Calculated from total plasma factor XI remaining divided by total factor XI coagulant activity times 100.
the platelet concentrate originated from the platelets and not from plasma contamination. The small amount of factor XI bound to the platelets after washing may indicate that some of our platelets were activated, since it has been shown that thrombin-activated platelets in the presence of high molecular weight kininogen bind factor XI. Similar experiments with labeled albumin indicated that 0.008% labeled albumin remained in the washed platelet suspension. These results also suggest that our platelet suspensions contained little plasma contamination.

Platelet Immunofluorescence Studies

Washed human platelet suspensions were examined for the presence of factor XI antigen by means of indirect immunofluorescence. Purified antibody to human plasma factor XI was used throughout the study. Rabbit control serum and factor XI immune serum absorbed with plasma factor XI did not stain the cells, whereas factor XI immune serum stained cells (Fig. 3). In addition, fluorescein-labeled goat anti-rabbit IgG and factor XI immune serum absorbed with factor X1 antibody (C). After the various treatments, samples were stained with FITC goat anti-rabbit IgG and viewed at 200 magnification with an Olympus fluorescence microscope.

![Fig. 3. Immunofluorescence of washed platelets. Platelets were incubated with control serum (A), with factor XI antibody absorbed with factor XI (B), or with factor XI antibody (C). After the various treatments, samples were stained with FITC goat anti-rabbit IgG and viewed at 200 magnification with an Olympus fluorescence microscope.](image)

Table 2. Immunofluorescence of Washed Human Cells

<table>
<thead>
<tr>
<th>Cell Preparation</th>
<th>Antisera</th>
<th>Fluorescence</th>
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<tbody>
<tr>
<td>Normal platelets</td>
<td>Anti-XI</td>
<td>+</td>
</tr>
<tr>
<td>Patient platelets*</td>
<td>Anti-XI</td>
<td>+</td>
</tr>
<tr>
<td>Red cells</td>
<td>Anti-XI</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoblasts</td>
<td>Anti-XI</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Anti-XI</td>
<td>0</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Anti-XI</td>
<td>0</td>
</tr>
<tr>
<td>Normal platelets</td>
<td>Anti-VIII</td>
<td>+</td>
</tr>
<tr>
<td>Normal platelets</td>
<td>Antifibrinogen</td>
<td>+</td>
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*Platelets from three patients with no detectable plasma factor XI were examined (see text for details).

Table 3. The Effect of Factor-XI-Deficient Plasma on Inhibition of Factor XI Activity by Anti-XI Antibody

<table>
<thead>
<tr>
<th>Plasma*</th>
<th>Clotting Times (sec) in FXI-Deficient Plasma</th>
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<tr>
<td></td>
<td>Anti-XI</td>
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<tr>
<td>FXI deficient†</td>
<td>842 (0%)</td>
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<tr>
<td>Normal‡</td>
<td>236 (100%)</td>
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<tr>
<td>Normal + FXI deficient</td>
<td>238 (96%)</td>
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*Samples were incubated for 30 min at 37°C before analysis. †1/10 dilution of factor-XI-deficient plasma. ‡1/50 dilution of normal plasma. §1/3750 dilution of anti-factor-XI antibody. The values in parentheses are percent of factor XI clotting activity. Either a 1/10 dilution of factor-XI-deficient plasma, a 1/50 dilution of normal plasma or a solution containing both a 1/10 dilution of factor-XI-deficient plasma and a 1/50 dilution of normal plasma were incubated in HEPES-buffered Tyrode's solution, pH 7.3, containing 1 mg/ml BSA at 37°C for 30 min with a 1/3750 dilution of anti-factor-XI antibody. Samples were then assayed for factor XI activity in a one-stage kaolin-activated clotting assay as described in Methods.
MW $\times 10^{-3}$

<table>
<thead>
<tr>
<th>MW</th>
<th>200-</th>
<th>130-</th>
<th>96-</th>
<th>68-</th>
<th>42-</th>
<th>30-</th>
<th>21-</th>
</tr>
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<td>2</td>
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**Fig. 4.** Autoradiograms of a 10% and 6% polyacrylamide SDS-gel of Staph A immunoprecipitates of radiolabeled platelet extracts and purified plasma factor XI. Thirty-five microliters of extract was applied to each well either containing 5% beta-mercaptoethanol or not. Each gel was run for 3 hr at 30 mA, stained with Coomassie blue, pressed onto paper, and subjected to autoradiography for 24 hr using intensifying screens. Twenty microcuries of platelet extract was immunoprecipitated. (A) All samples reduced and run on a 10% gel. Lane 1, immunoprecipitate of extract using normal rabbit serum; lane 2, total platelet extract (50,000 cpm); lane 3, immunoprecipitate of platelet extract using anti-factor-XI antibody; lane 4, immunoprecipitate of labeled purified factor XI (100,000 cpm); lane 5, immunoprecipitate of platelet extract using anti-human albumin. (B) All samples unreduced and run on a 6% gel. Lane 1, immunoprecipitate of platelet extract using control serum; lane 2, immunoprecipitate of platelet extract using anti-factor-XI antibody; lane 3, immunoprecipitate of purified plasma factor XI using anti-factor-XI antibody. Molecular weight standards were purchased from Bio-Rad and were myosin, 200,000; beta-galactosidase, 130,000; phosphorylase B 96,000; albumin, 68,000; ovalbumin, 42,000; carbonic anhydrase, 30,000; and lysozyme, 20,000.

Front. In contrast, purified factor XI immunoprecipitated with anti-factor-XI antibody shows a single band at 80,000 daltons under reducing conditions (lane 4). Total platelet extract that was subjected to immunoprecipitation does not show a major band at 52,000 daltons, suggesting that platelet XI is a trace component of our platelet extracts. Immunoprecipitates of control serum (lane 1) reveal little radioactivity, and immunoprecipitates of anti-albumin reveal the expected band at 68,000 daltons (lane 5), indicating that our immunoprecipitation procedure shows specificity. Under nonreducing conditions, immunoprecipitates of the platelet material give a molecular weight of 220,000 daltons (Fig. 4B, lane 2) and those of purified plasma factor XI give a molecular weight of 160,000 daltons (Fig. 4B, lane 3). Immunoprecipitation experiments with platelets obtained from a patient with no detectable plasma factor XI showed results identical to normal platelets (data not shown).

These results indicate that platelet factor XI, although immunologically related to plasma factor XI, differs from it in structure. These data are consistent with the interpretation that platelet factor XI is a disulfide-linked tetramer of molecular weight 220,000 daltons.

**Concentration and Further Characterization of Platelet Factor XI**

In an effort to further characterize and establish the molecular characteristics of platelet-associated factor XI, a procedure was adopted that both enriched for factor XI and defined some of its properties. Platelets were washed, extracted with Triton X-100, and the extracts absorbed on Con-A-Sepharose (see Materials and Methods). Proteolytic enzyme inhibitors were added to the platelet suspensions prior to lysis with Triton. The Con-A-Sepharose column was eluted with 1.0 M $\alpha$-methylmannoside. The eluate was concentrated, subjected to immunoelectrophoresis, and analyzed for factor XI antigen and activity. This platelet
preparative procedure yielded 100 μg of platelet factor XI antigen as measured by radial immunodiffusion.

Purified plasma factor XI was coelectrophoresed with platelet Con-A extract (Fig. 5). The inclusion of proteolytic enzyme inhibitors in our Triton extracts precluded the measurement of functional activity. Both plasma and platelet factor XI samples migrated toward the negative electrode with the platelet material showing a slightly lower cathodal mobility. However, the “gullwing” configuration observed when platelet and plasma factor XI were coelectrophoresed is evidence of immunologic identity. The difference in mobility could not be altered by reducing agents. Preparations of plasma factor XI containing 20% zymogen and 80% factor Xla gave immunoelectrophoretic results identical to preparations of plasma factor XI with no detectable factor Xla activity. Therefore, it is unlikely that the differences in mobility between platelet and plasma factor XI in these experiments is due to proteolytic activation of factor XI.

To investigate the possibility that the lower cathodal mobility of the platelet factor XI could be due to complex formation with high molecular weight (HMW) kininogen, purified plasma factor XI and HMW kininogen were incubated in molar ratios of factor XI to HMW kininogen of 1, 2, and 10, and the incubation mixtures were subjected to immunoelectrophoresis. Incubations were carried out at room temperature for 5 min in HEPES-buffered Tyrode’s containing 0.1% bovine serum albumin. Final concentrations of factor XI were 0.2 μM (8 U/ml) and the HMW kininogen concentration in the incubation mixtures was varied from 0.2 to 2 μM. There was no detectable anodal shift of the electrophoretograms of these incubation mixtures (data not shown), suggesting that complex formation could not account for the differences in electrophoretic mobility between platelet factor XI and plasma factor XI in our system.

To investigate the possibility that proteolysis was altering factor XI in our platelet extracts, labeled purified plasma factor XI was added to a Triton extract of platelets containing enzyme inhibitors (see Materials and Methods) and incubated for 3 hr at 37°C. The gel profiles of the purified factor XI incubated in the platelet Triton extract and that of the control were identical (data not shown). Thus, the platelet extract containing proteolytic inhibitors did not cause significant proteolysis of factor XI.

The electrophoresed platelet extract was analyzed by clotting eluted portions of an identical concurrently run agarose gel (Fig. 6). Factor XI activity was detected in a region of the gel that coincided with the anti-factor-XI precipitin arc. Similar results were obtained with purified factor XI (data not shown). No factor Xla activity was detected in this region of the gel, i.e., preincubation with kaolin was needed for expression of activity. This region of the gel contained no detectable factor V activity.

A Triton extract of platelets containing no proteolytic enzyme inhibitors was passed over a Con-A column and the inhibition of the factor XI activity of the column eluate by factor XI antibody measured. As can be seen from Table 4, the factor XI clotting activity of the Triton extract before passage over the column could not be inhibited with anti-factor-XI antibody, whereas the column eluate was readily inhib-
Table 4. The Effect of Anti-Factor-XI Antibody on Platelet and Plasma Factor XI Activities

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Units Factor XI/Liter</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-XI</td>
<td>Anti-XI†</td>
</tr>
<tr>
<td>Triton extract of platelet factor XI</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Platelet factor XI purified on Con-A</td>
<td>4.2</td>
<td>&lt;0.10†</td>
</tr>
<tr>
<td>Purified plasma factor XI</td>
<td>4.0</td>
<td>&lt;0.10†</td>
</tr>
</tbody>
</table>

*Fifty-three units of outdated platelet concentrates were extracted with 0.2% Triton as described in Methods except that proteolytic enzyme inhibitors were omitted.
†Samples were incubated for 10 min at 37°C with a 1/100 dilution of anti-factor-XI antibody.
‡Clotting times were equivalent to the value of the blank that was 335 sec.

The existence and physiologic relevance of platelet factor XI has been a matter of controversy since Walsh’s first postulated that washed platelets activated with collagen contain a factor XI activity that can participate in the initiation of intrinsic blood coagulation. These early studies were based on the observation that normal collagen-treated platelets could correct the clotting defect of factor-XII-deficient as well as factor-XI-deficient plasmas. In contrast, partially purified platelet factor XI was inhibited by antibody.

DISCUSSION

The nature of factor XI in platelets was also at least tentatively established by two independent procedures: (1) Immunoelectrophoresis experiments indicate that platelet factor XI appears to be less basic and shows complete identity with plasma factor XI (Figs. 5 and 6); (2) immunoprecipitation experiments indicate that platelet factor XI is composed of a 52,000 molecular weight subunit on reduced gels (Fig. 4A) and runs as a protein of molecular weight approximately 220,000 on unreduced gels (Fig. 4B). These results strongly suggest that platelet factor XI is immunologically related to plasma factor XI but structurally distinct. The differences observed could not be due to nonspecific proteolysis of the platelet factor XI during solubilization despite the presence of proteolytic enzyme inhibitors, since platelet factor XI is larger than plasma factor XI. In addition, plasma factor XI incubated for 3 hr at 37°C in platelet extract containing inhibitors appeared to maintain its structure and activity (data not shown). Another possible explanation for the differences in electrophoretic mobility of plasma and platelet factor XI could be due to complex formation with high molecular weight kininogen or with α,-antitrypsin, which has been reported to inhibit factor XI and is known to be present in platelets. It is unlikely that these results represent complex formation with α,-antitrypsin, since the factor XI found in platelets before washing and then measured after washing indicated that at least 90% of plasma factor XI was removed. The amount of factor XI activity remaining in washed platelet concentrates was tenfold higher than could be accounted for by plasma factor XI contamination. These data clearly demonstrate that nearly all of the factor XI in our platelet suspensions originates from platelets and therefore must be in a form that is not readily exchangeable with exogenous plasma factor XI.

The presence of factor XI in our washed platelet preparations was demonstrated by four independent techniques:

1. Factor XI clotting assays indicate 0.67 U of activity per 10¹¹ platelets.
2. Immunofluorescence experiments clearly demonstrate the presence of factor XI antigen in normal platelets as well as platelets from patients deficient in plasma factor XI (Fig. 3).
3. Immunoelectrophoresis experiments (Figs. 5 and 6) demonstrate that both platelet factor XI antigen and activity coelectrophorese, although this experiment does not prove that factor XI antigen in our preparations gives rise to factor XI activity, since our platelet preparations contain numerous proteins.
4. Immunoprecipitation experiments (Fig. 4) demonstrate the presence of factor XI antigen in extracts of washed platelets.

Plasma contamination in our platelet preparations was assessed by two independent criteria. Labeled factor XI and bovine serum albumin added to our
let is in zymogen form and $\alpha_1$-antitrypsin inactivates only factor Xa.\textsuperscript{36} Furthermore, this would not explain the molecular weights observed in the immunoprecipitation experiments (Fig. 4). A complex of factor XI with high molecular weight kininogen would be expected to have a lower isoelectric point and have a lower cathodal mobility. Addition of purified high molecular weight kininogen to purified plasma factor XI in stoichiometric ratios of 1:1, 2:1, and 10:1 had no effect on the cathodal mobility of purified factor XI, indicating that under our incubation and electrophoretic conditions, complex formation with high molecular weight kininogen could not be detected. This does not rule out endogenous complex formation between platelet factor XI and platelet kininogen.\textsuperscript{33} However, a platelet high molecular kininogen factor XI complex would not account for the molecular weight distributions shown in Fig. 4, and the complex would most likely have to be covalent, since boiling in SDS and urea did not dissociate it.

Our studies indicate that platelets from three hemostatically normal patients with no detectable plasma factor XI had factor XI antigen detected by immunofluorescence and factor XI activity in coagulation assays. Patients with factor XI deficiency vary considerably in clinical severity, and this result is consistent with the interpretation that platelet factor XI can substitute for plasma factor XI in hemostasis and account for this clinical variability. However, other interpretations are possible. Furthermore, validation of any such relationship would require the demonstration that patients with plasma factor XI deficiency and bleeding complications have less platelet factor XI antigen than hemostatically normal patients deficient in plasma factor XI only. This would require the development of a quantitative method for determination of platelet-associated factor XI antigen.

The studies presented here help to clarify previous observations\textsuperscript{3} about the origin and nature of platelet factor XI. It was previously shown that the platelet factor XI activity, which resides in the membrane fraction, is not inactivated by anti-factor-XI antibody and is present in the platelets from a hemostatically normal patient with no detectable factor XI. It was suggested that the "platelet factor-XI-like activity arises from a molecule similar or identical to plasma factor XI situated in the plasma membrane, so as to protect it from inhibition by anti-factor-XI antibody.\textsuperscript{39}"

Our present studies support the view that platelet factor XI is functionally and antigenically similar to plasma factor XI but different in molecular weight and isoelectric point. The failure of anti-factor-XI antibody to inactivate the intrinsic platelet factor XI may indeed be a result of its orientation in the membrane and association with membrane proteins, since when the platelet factor XI is made soluble by detergent lysis and partially purified on Con-A–Sepharose, its activity is inhibited by antibody (Table 4). In addition, the presence of factor XI activity and antigen in the platelets of three hemostatically normal individuals with no detectable plasma factor XI activity or antigen supports the conclusion that the platelet material is not of plasma origin and that it may substitute for plasma factor XI in hemostasis. The ultimate proof, however, that platelet factor XI can substitute for plasma factor XI must await experiments that demonstrate the ability of the platelet material to cleave factor IX in an in vitro assay system. Such experiments are currently underway.

**ACKNOWLEDGMENT**

The authors would like to thank B. White and E. Trinkner for their skillful technical assistance; Dr. Linda Knight for radiolabeling the Con-A extracts and factor XI preparations; and Dr. Margaret Johnson for referring two factor-XI-deficient patients.

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Factor XI antigen and activity in human platelets

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