Role of high-molecular-weight kininogen in surface-binding and activation of coagulation Factor XI and prekallikrein

(Hageman factor/contact activation/fibrinolysis)

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ABSTRACT In the contact phase of activation of the kinin-forming, intrinsic clotting, and fibrinolytic systems, high-molecular-weight kininogen acts as a cofactor for the activation of Factor XI, prekallikrein, and Hageman factor. One mechanism by which high-molecular-weight kininogen acts as a cofactor has been studied by using 125I-labeled Factor XI and prekallikrein in kaolin-activated normal human plasma and plasmas deficient in high-molecular-weight kininogen and Hageman factor. High-molecular-weight kininogen was found to be essential for normal binding and cleavage of both Factor XI and prekallikrein on the kaolin surface. Hageman factor was essential for cleavage but not for binding of Factor XI and prekallikrein to kaolin. In normal plasma 80% of the activated Factor XI remained surface-bound, whereas 80% of the kalli- kinin was not surface-bound. These findings are consistent with the hypothesis that, in the initial phase of contact activation, high-molecular-weight kininogen links both Factor XI and prekallikrein to the exposed surface where they are activated by surface-bound activated Hageman factor. Once activated, the Factor XI molecules remain localized at the site of activation, in contrast to the kallikrein molecules which are found largely in the surrounding plasma.

Activation of Hageman factor (Factor XII) upon a negatively charged surface initiates intrinsic coagulation, fibrinolysis, and the generation of vasoactive peptides (1–5). High-molecular-weight (Mr) kininogen is a cofactor for the optimal activation of surface-bound Hageman factor by kallikrein (6–8) and for the activation of prekallikrein and Factor XI by surface-bound activated Hageman factor (6–8). Prekallikrein is complexed to high Mr kininogen in plasma (9). Recent studies (10; R. C. Wiggins, B. N. Bouma, C. G. Cochrane, and J. H. Griffin, unpublished data) suggest that Factor XI is also complexed to high Mr kininogen in plasma. In this study we demonstrate that one mechanism by which high Mr kininogen acts as a cofactor for the activation of both Factor XI and prekallikrein in plasma is to bind both these molecules to the surface where they are activated by surface-bound activated Hageman factor. We also demonstrate that, while activated Factor XI remains associated with the surface, kallikrein dissociates from the surface.

MATERIALS AND METHODS

Purified proteins

Factor XI was isolated from normal human plasma as described elsewhere (11) and was determined to be more than 95% homogeneous on sodium dodecyl sulfate (NaDodSO4)/polyacrylamide gels. The purified protein had a specific clotting activity of 220 units/mg, where 1 clotting unit is defined as the amount of activity present in 1 ml of citrated normal human plasma. Factor XI was radiolabeled with 125I-iodine by the insolubilized lactoperoxidase method (12). The 125I-labeled Factor XI (125I-Factor XI), 5 μCi/μg, retained its procoagulant activities at 3.6 clotting units/ml.

Prekallikrein was isolated from normal human plasma by a method similar to that described for Factor XI (11). This involved the chromatography of prekallikrein on DEAE-, QAE-, and SP-Sephadex, and on concanavalin A-Sepharose, and sucrose density ultracentrifugation (B. N. Bouma and J. H. Griffin, unpublished data). The protein was more than 95% homogeneous on NaDodSO4/polyacrylamide gels. The purified protein had a specific clotting activity of 19 units/mg, where 1 clotting unit is defined as the amount of activity present in 1 ml of citrated normal human plasma. Prekallikrein was radiolabeled with 125I-iodine by the chloramine-T method (13); after radiolabeling 12% of the material appeared as kallikrein. Only the heavy chain (45,000 Mr) was radiolabeled by the above procedure; therefore the light chain (35,000 Mr) containing the active site (and which could bind to inhibitors in plasma) was not seen on reduced NaDodSO4/polyacrylamide gels. The 125I-labeled prekallikrein (125I-prekallikrein), 3 μCi/μg, retained its procoagulant activity at 2.3 clotting units/ml.

High Mr Kininogen was isolated from freshly prepared normal human plasma containing acid/citrate/dextrose anticoagulant (1 liter contains 13.6 g of citric acid, 25 g of sodium citrate, and 20 g of dextrose). Plasma (1.4 liters) was chromatographed on DEAE-Sephadex exactly as described for the isolation of Hageman factor (14). The pool of fractions containing activity that corrected the clotting defect of plasma deficient in high Mr kininogen was brought to 47% saturation of (NH4)2SO4 by addition of saturated (NH4)2SO4 at 4°C. After the mixture was stirred for 1 hr, the precipitate was collected by centrifugation at 5600 × g for 90 min. The precipitate was dissolved in 100 ml of buffer containing 50 mM acetate, 75 mM NaCl, 0.5 mM EDTA, 0.5 mM benzamidine, 25 μg of Polybrene per ml, 0.01% sodium azide, at pH 5.3, and the sample was applied to an SP-Sephadex column which was developed exactly as described as step 5 in the isolation of Hageman factor (14). The high Mr kininogen, which was eluted in the terminal region of the peak of activity from this column, was greater than 90% homogeneous as judged on NaDodSO4 gels, and it exhibited a specific clotting activity of 12 ± 2 clotting units/mg of protein.

Deficient plasma

Normal human plasma was made from a pool of blood from 15 normal adults. Hageman factor-deficient plasma was generated by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1794 solely to indicate this fact.

Abbreviations: high Mr, kininogen, high-molecular-weight kininogen; 125I-protein, 125I-labeled protein; NaDodSO4, sodium dodecyl sulfate.

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Calculation of the method

mM), and containing (20, ul) (10%)

pellet

2

 added

of

undiluted

plasma

Mr

high

Mr

(0.14, ug,

Binding

XI

125I-Factor

Biomedical.

(Fitzgerald

plasma

ously donated by Gunda Hiatt. High Mr kininogen-deficient plasma (Fitzgerald trait) was purchased from George King Biomedical.

Binding studies

125I-Factor XI (0.03 μg, 0.15 μCi in 2 μl) or 125I-prekallikrein (0.14 μg, 0.13 μCi in 2 μl) was added to 30 μl of a 1:1 dilution of plasma in Tris-buffered saline. The plasmas used were normal human plasma, Hageman factor-deficient plasma, high Mr kininogen-deficient plasma, and high Mr kininogen-deficient plasma plus purified high Mr kininogen.

50,000, and 33,000 Mr peaks for Factor XI and the 85,000 and 48,000 Mr peaks for prekallikrein from the kaolin and from the supernatant. A volume correction was made for the aliquot from the supernatant. The proportions of cleaved proteins were estimated by calculating the percentage of the total radioactivity present in the 50,000 plus 33,000 Mr peaks for Factor XI and the 48,000 Mr peak for prekallikrein. Correspondingly, the percentage of each protein that was not cleaved was calculated as the percentage of total radioactivity in the 83,000 or 85,000 Mr peak for Factor XI or prekallikrein, respectively. Finally, in control gels when no kaolin had been added (which gave profiles identical to those in Figs. 1B and 2B) the proportion of radioactivity corresponding to the position of the cleavage fragments was calculated and found to be 5% for 125I-Factor XI and 12% for 125I-prekallikrein. These corrections were subtracted from the calculated cleavage values to give the figures presented in Tables 1 and 2. More than 60% of the 125I-iodine added to the plasma was recovered on the polyacrylamide gels.

RESULTS

Factor XI

The binding and cleavage of 125I-Factor XI in kaolin-activated normal plasma and in plasmas deficient in Hageman factor and high Mr kininogen are shown in Fig. 1 and Table 1. Whereas in normal plasma after a 2-min incubation at 37°, 87% of the Factor XI was bound to kaolin (Fig. 1A), in the absence of high Mr kininogen only 14% was bound (Fig. 1C). When high Mr kininogen-deficient plasma was reconstituted by addition of purified high Mr kininogen, the binding of 125I-Factor XI to

Calculation of binding and cleavage data

The total bound and free 125I-Factor XI and 125I-prekallikrein was calculated by integrating the radioactivity in the 83,000,
kaolin increased to 79% (Fig. 1D). In the absence of Hageman factor, binding was slightly reduced at 66% (Fig. 1B). High \( M_r \) kininogen, but not Hageman factor, is therefore necessary for the efficient binding of Factor XI to the kaolin surface.

Most of the Factor XI bound to kaolin (96%) and in the supernatant (62%) was cleaved in normal plasma. However, in both Hageman factor-deficient and high \( M_r \) kininogen-deficient plasma minimal cleavage of Factor XI occurred. When the high \( M_r \) kininogen-deficient plasma was reconstituted with purified high \( M_r \) kininogen, cleavage of the bound Factor XI occurred normally. These results suggest that both high \( M_r \) kininogen and Hageman factor are required for efficient cleavage of Factor XI in plasma.

Experiments conducted over shorter incubation periods at 20° and longer incubation periods at 37° gave substantially similar results, showing that high \( M_r \) kininogen was required for normal binding and cleavage levels to be attained, and that once bound, the \( ^{125}\)I-Factor XI remained attached to the kaolin.

### Prekallikrein

The binding and cleavage of \( ^{125}\)I-prekallikrein in kaolin-activated normal plasma and plasmas deficient in Hageman factor and high \( M_r \) kininogen are shown in Fig. 2 and Table 2. After a 2-min incubation at 20° in normal plasma most of the prekallikrein was cleaved both on the kaolin (64%) and in the supernatant (62%) (Fig. 2A). In the absence of high \( M_r \) kininogen (Fig. 2C) and Hageman factor (Fig. 2B) cleavage was minimal (i.e., less than 0.5%), whereas upon reconstitution of the high \( M_r \) kininogen-deficient plasma with purified high \( M_r \) kininogen, cleavage occurred normally (Fig. 2D). Therefore, both high \( M_r \) kininogen and Hageman factor are required for the efficient cleavage of prekallikrein in plasma.

In high \( M_r \) kininogen-deficient plasma only 2% of the \( ^{125}\)I-prekallikrein was bound to kaolin in contrast to 14% bound in normal plasma. Reconstitution of high \( M_r \) kininogen-deficient plasma with purified high \( M_r \) kininogen increased the binding to normal levels.

Experiments conducted over longer incubation periods up to 10 min at 37° gave substantially similar results.

### DISCUSSION

Human plasma deficient in high \( M_r \) kininogen exhibits gross abnormalities in contact activation reactions (16–20). The functional role of high \( M_r \) kininogen as a cofactor in the activation of Hageman factor by kallikrein and in the activation of Factor XI or prekallikrein by surface-bound activated Hageman factor was subsequently demonstrated in studies using purified proteins (6–8). In the present study the role of high \( M_r \) kininogen in the binding of \( ^{125}\)I-Factor XI and \( ^{125}\)I-prekallikrein to kaolin in the milieu of plasma was investigated. In addition, the limited proteolytic cleavage that is coincident with activation of the two molecules (11, 21) was studied in normal plasma and in plasmas deficient in high \( M_r \) kininogen and Hageman factor. Previous studies utilizing \( ^{125}\)I-Hageman factor added to normal plasma and plasmas deficient in high \( M_r \) kininogen and Hageman factor have provided new insight into the binding and proteolytic activation of Hageman factor during contact activation (22).

The experiments with \( ^{125}\)I-Factor XI demonstrate that high \( M_r \) kininogen is required for normal extensive binding of Factor XI to kaolin in plasma. The fact that the addition of purified high \( M_r \) kininogen to the kininogen-deficient plasma reconstituted the binding of \( ^{125}\)I-Factor XI to kaolin shows that the binding abnormality is directly related to high \( M_r \) kininogen and not to an inhibitor present in the deficient plasma. Since in Hageman factor-deficient plasma the binding of \( ^{125}\)I-Factor XI to kaolin is extensive although there is no proteolytic cleavage of Factor XI, we conclude that high \( M_r \) kininogen mediates the binding of unactivated Factor XI to kaolin. This suggests that the formation of surface-bound complexes of Factor XI and high \( M_r \) kininogen may occur before the activation of Factor XI and, indeed, may be a prerequisite for efficient activation of Factor XI. Fig. 3 summarizes four hypothesized reactions that involve Factor XI and high \( M_r \) kininogen. The first reaction, a reversible equilibrium for which the equilibrium constant is unknown, describes complex formation in plasma between the two molecules. The reaction is supported by studies of these two molecules in plasma analyzed by Sephadex G-200 chromatography (ref. 10; R. C. Wiggins, B. N. Bouma, C. G. Cochrane, and J. H. Griffin, unpublished

### Table 1. Binding and cleavage of \( ^{125}\)I-Factor XI in various plasmas

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Kaolin-bound, %</th>
<th>Supernatant, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Cleaved</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>Hageman factor-deficient plasma</td>
<td>66</td>
<td>&lt;1</td>
</tr>
<tr>
<td>High ( M_r ) kininogen-deficient plasma</td>
<td>14</td>
<td>&lt;2</td>
</tr>
<tr>
<td>High ( M_r ) kininogen-deficient plasma + purified high ( M_r ) kininogen</td>
<td>79</td>
<td>61</td>
</tr>
</tbody>
</table>

The binding and cleavage of \( ^{125}\)I-prekallikrein in various plasmas after addition of kaolin and incubation for 2 min at 20°. The kaolin-bound material and supernatant were separated, reduced, and analyzed in 7.5% NaDodSO\(_4\)/polyacrylamide gels. The values given are derived from integration of radiolabeled prekallikrein in the 85,000 \( M_r \), uncleaved molecule and the 48,000 \( M_r \), cleavage fragment shown in Fig. 2.
FACTOR XI
HMWK
COMPLEX

FACTOR XI +
HMWK

SURFACE

FACTOR XI
HMWK-SURFACE

SURFACE-BOUND
ACTIVATED HAGEMAN
FACTOR

FACTOR XI
HMWK-SURFACE

FIG. 3. Schematic role for high Mr kininogen (HMWK) in surface-binding and activation of Factor XI in plasma.

FACTOR XI +
HMWK-SURFACE

FIG. 4. Schematic role for high Mr kininogen (HMWK) in surface-binding and activation of prekallikrein in plasma.
M, kininogen corrected the binding abnormality found in high M, kininogen-deficient plasma. Hence, high M, kininogen mediates the efficient binding of prekallikrein to kaolin. Fig. 4 summarizes four reactions that involve high M, kininogen and prekallikrein. The first reaction depicts a reversible complex formation in plasma between the two molecules for which the equilibrium constant in unknown (9, 25, 26). The second reaction involves the binding of this bimolecular complex to kaolin; the notation is meant to imply that the kininogen molecule bridges or links prekallikrein to kaolin in plasma, as suggested above for Factor XI. In the third reaction surface-bound activated Hageman factor cleaves prekallikrein to give the active enzyme kallikrein, as suggested previously (6). The fourth reaction is the dissociation of surface-bound kallikrein. The final equilibrium in favor of dissociation is consistent with the fact that 80% of the kallikrein is free in solution, and repetitive washing studies showed that kallikrein could be eluted from a glass surface (S. D. Revak, R. C. Wiggins, B. N. Bouma, C. G. Cochrane, and J. H. Griffin, unpublished data). However, this does not preclude the possibility of activation of prekallikrein in solution, a reaction that is readily catalyzed by a 28,000 M, form of activated Hageman factor (27, 28).

In normal plasma after contact activation the observations summarized in Tables 1 and 2 show that 5 out of 6 molecules of kallikrein are in solution whereas 9 out of 10 molecules of activated Factor XI are surface-bound. If this distribution of activated enzymes were to occur upon contact activation in vitro after injury and exposure of damaged connective tissue (29), collagen (30–32), or basement membrane structures (33), it would suggest that coagulation reactions might tend to remain localized while kallikrein-dependent reactions would occur in solution away from the site of injury. This implication must be tempered by the possible differences in specific enzyme activities or susceptibility to inhibition of soluble versus surface-bound enzymes.

Harpel (34) showed that partially purified kallikrein would bind to collagen and that, after brief incubation of particulate collagen with undiluted plasma, kallikrein activity could be identified on the washed collagen. Assuming that collagen and kaolin both act as surfaces with negative changes, the binding of kallikrein to collagen observed by Harpel could represent the limited binding that occurred in the absence of high M, kininogen observed in the present study.

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