# Association of Factor XI and High Molecular Weight Kininogen in Human Plasma

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ABSTRACT Factor XI and high molecular weight kininogen were found associated in normal human plasma at mol wt 380,000 as assessed by gel filtration on Sephadex G-200. The molecular weight of Factor XI in high molecular weight kininogen-deficient plasma was 175,000, the same value obtained for purified Factor XI. When high molecular weight kininogen-deficient plasma was reconstituted with purified high molecular weight kininogen, all of the Factor XI was then found at mol wt 380,000. Complex formation was also demonstrable upon incubation of Factor XI and highly purified high molecular weight kininogen. This complex was distinct from the prekallikrein-high molecular weight kininogen complex; thus high molecular weight kininogen forms bimolecular complexes with either Factor XI or prekallikrein but does not form a trimolecular complex that includes both Factor XI and prekallikrein. Neither Hageman factor nor plasminogen were found associated with high molecular weight kininogen; binding to high molecular weight kininogen appeared to be a specific property of the Hageman factor substrates.

## INTRODUCTION

Each protein of the plasma kinin-forming system has been shown to function as a coagulation factor. Thus, plasmas that are deficient in Hageman factor, prekallikrein, or high molecular weight (HMW)[1] kininogen exhibit a prolonged partial thromboplastin time which reflects their relative rate of Hageman factor activation (1–9). HMW kininogen has been shown to function as a cofactor in the activation of prekallikrein by Hageman factor as well as the activation of Hageman factor by kallikrein (10–14). We have recently demonstrated that prekallikrein and HMW kininogen normally circulate as a complex (15) and proposed that such binding might facilitate the trimolecular interaction that takes place upon the surface. Because the activation of Factor XI, the other Hageman factor substrate, is also augmented by HMW kininogen (10–13, 16), we have examined the possibility that Factor XI forms a complex with HMW kininogen (17). In this paper we show that Factor XI is bound to HMW kininogen in whole plasma and that such binding is also demonstrable utilizing highly purified components.

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[1] *Abbreviation used in this paper:* HMW, high molecular weight.

## METHODS

Apoferritin and catalase (Calbiochem, San Diego, Calif.); blue dextran, ovalbumin, chymotrypsinogen, QAE-Sephadex, SP-Sephadex, CM-Sepharose, and Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.); human crystallized albumin (Sigma Chemical Co., St. Louis, Mo.); and rabbit antibody to human plasminogen (Behring Diagnostics, American Hoechst Corp., Somerville, N.J.) were obtained as indicated. Plasma, deficient in Hageman factor or Factor XI, and containing 0.38% sodium citrate was obtained from Sera Tec Biologicals, New Brunswick, N.J. Kininogen-deficient plasma obtained from Ms. Williams was a gift from Dr. Robert W. Colman (University of Pennsylvania, Philadelphia, Pa.). Urokinase was kindly provided by Dr. Grant Barlow (Abbott Laboratories, Diagnostics Div., Chicago, Ill.).

*Preparation of plasma proteins.* Fresh plasma used for the isolation of Factor XI and HMW kininogen was collected in 0.38% sodium citrate. Hexadimethrine bromide (3.6 mg) in 0.1 ml of 0.15 M saline was added for each 10 ml of blood drawn. The tubes were then centrifuged at 900 *g* for 20 min at 4°C, and the plasma was separated with plastic pipettes. Plastic columns and test tubes were used throughout to minimize activation of Hageman factor and nonspecific adsorption to glass surfaces. Samples of Factor XI were concentrated by ultrafiltration (Amicon Corp., Lexington, Mass.) through a UM-10 membrane whereas fractions containing HMW kininogen were lyophilized.

Gel filtration on Sephadex G-150 (18), alkaline disc gel electrophoresis (19), and sodium dodecyl sulfate gel electrophoresis (20) were performed as described.

*Factor XI.* Factor XI was isolated by a modification of the method of Heck and Kaplan (21), however the final immunoadsorption to remove IgG was omitted. 2 liters of fresh plasma were dialyzed against 0.003 M $PO_4$ buffer (pH 8.3)

and passed over a 20 × 100-cm column of QAE-Sephadex equilibrated with the same buffer. The effluent, containing a mixture of proteins of gamma globulin mobility was then fractionated by sequential chromatography on SP-Sephadex and Sephadex G-150. In the gel filtration experiments described herein Factor XI was utilized after the Sephadex G-150 step which prevented any aggregation of Factor XI (observed upon recycling purified Factor XI) and the IgG was followed as an internal marker. The Factor XI preparation contained 5.0 U Factor XI/ml if 1 U is defined as the concentration of Factor XI in normal human plasma. It contained no detectable Hageman factor, prekallikrein, HMW kininogen, or plasminogen when assayed at a concentration of 25 U/ml.

*HMW kininogen.* HMW kininogen was isolated from 2 liters of normal plasma by chromatography on QAE-Sephadex A-50, ammonium sulfate precipitation, fractionation on SP-Sephadex, and Sephadex G-200 gel filtration according to the method of Habal et al. (22). A small percentage of aggregated HMW kininogen was identified and only the major peak found at an apparent mol wt 210,000 was utilized (23).

The preparation was quantitated functionally by its ability to selectively correct the coagulation defect in Williams trait plasma (6) and it was free of any detectable Hageman factor, prekallikrein, or Factor XI. HMW kininogen protein was quantitated by the Lowry method (24) using human albumin as a protein standard. Its sp act was 12.5 U/mg protein if 1 U is defined as the coagulant activity of HMW kininogen in 1 ml of pooled normal human plasma. The preparation was routinely utilized at 4 mg/ml. Upon sodium dodecyl sulfate gel electrophoresis, a single band was obtained at an estimated mol wt 125,000 as shown in Fig. 1.

FIGURE 1 Sodium dodecyl sulfate gel electrophoresis of 20 μg purified HMW kininogen.

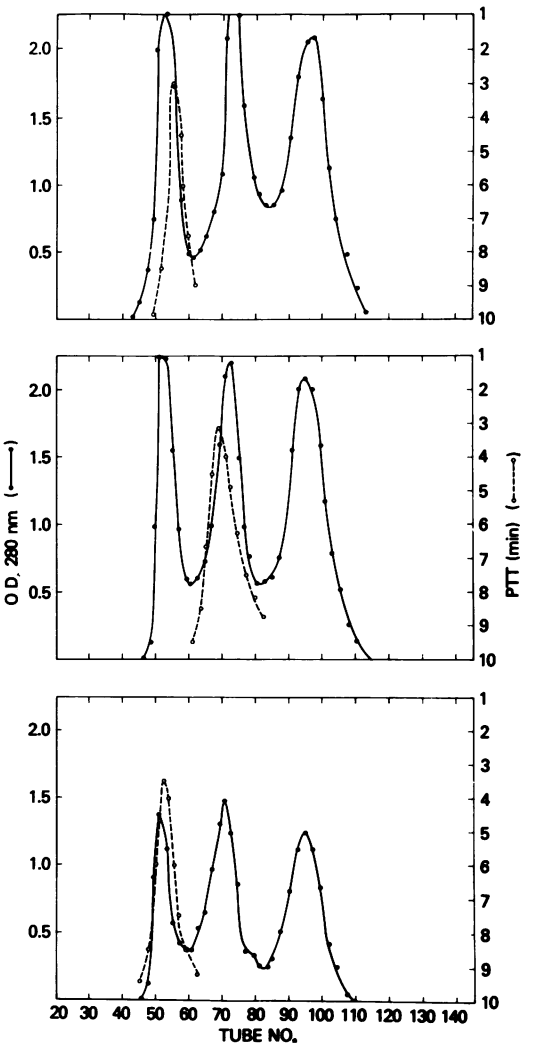


FIGURE 2 Sephadex G-200 gel filtration of 4.0 ml normal plasma (top panel), 4.0 ml of HMW kininogen-deficient plasma (center panel), and 4.0 ml of high molecular weight kininogen-deficient plasma after reconstitution with 80 μg/ml HMW (bottom panel). The elution profile of Factor XI is shown in each case.

*Coagulation assays.* Hageman factor, prekallikrein, and HMW kininogen were determined by a modification of the procedure for determining the partial thromboplastin time (25) utilizing congenitally deficient plasma.

*Plasminogen assay.* Plasminogen was assayed by incubating column fractions with 50 μg urokinase for 30 min at 37°C, and the plasmin generated determined by the fibrin plate assay (26). Antigenic plasminogen was sought by

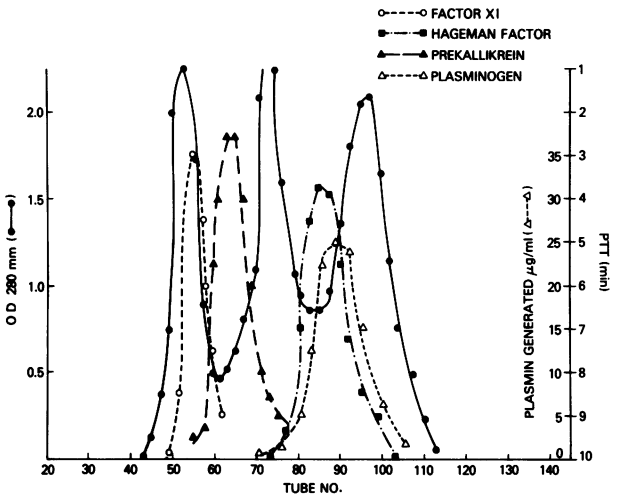


FIGURE 3 Sephadex G-200 gel filtration of 4.0 ml of normal plasma indicating the relative position of the Factor XI-HMW kininogen complex, the prekallikrein-HMW kininogen complex, Hageman factor, and plasminogen.

Ouchterlony analysis of column fractions (27) utilizing monospecific antibody to human plasminogen.

*Sephadex G-200 gel filtration.* A 2.6 × 95-cm Pharmacia K26/100 column of Sephadex G-200 was equilibrated with 0.01 M Tris-HCl buffer (pH 7.0) made 0.15 M in NaCl. The column was run at 4°C, the elution rate with upward flow was 10 ml/h, 4-ml samples of citrated plasmas (without Polybrene) or isolated proteins were applied, and 3.0-ml fractions were collected. Molecular weights were determined by gel filtration according to the method of Andrews (28). The standards used and their molecular weights by gel filtration were apoferritin (460,000), catalase (195,000), bovine serum albumin (65,000), and chymotrypsinogen (25,000).

## RESULTS

When normal human plasma was fractionated on Sephadex G-200 and assayed for Factor XI a peak of activity was found at ≅ mol wt 380,000 as shown in the upper panel of Fig. 2. When HMW kininogen-deficient plasma was similarly fractionated and assayed for Factor XI, the activity was found at mol wt 175,000 corresponding to the molecular weight reported upon gel filtration of purified Factor XI (21). (Fig. 2, center panel). When the HMW kininogen-deficient plasma was reconstituted with 80 µg HMW kininogen for each mililiter of plasma and then fractionated on Sephadex G-200, all of the Factor XI was again found at mol wt 380,000 (Fig. 2, lower panel). Thus, it appeared that Factor XI circulated as a complex with HMW kininogen. Because prekallikrein also circulates complexed to HMW kininogen, we next examined the possibility that a trimolecular complex is formed containing prekallikrein, Factor XI, and HMW kininogen. The functional assessment of each of these factors on a Sephadex G-200 chromatogram of normal plasma is shown in Fig. 3. Factor XI was found at mol wt 380,000 and prekallikrein was found at mol wt 285,000; there was no detectable peak of activity containing both Hageman factor substrates suggesting that they each bind to different molecules of HMW kininogen. Hageman factor was found at mol wt 115,000 indicating that it is not complexed to HMW kininogen (15). Because Factor XIa (29, 30), kallikrein (29), and plasmin (31) can each function as an activator of Hageman factor, the possibility that plasminogen circulates bound to HMW kininogen was also examined. When assessed functionally, all the plasminogen was found at mol wt 95,000, suggesting that there was no plasminogen-HMW kininogen complex. However, plasminogen is present in plasma at approximately three times the concentration of HMW

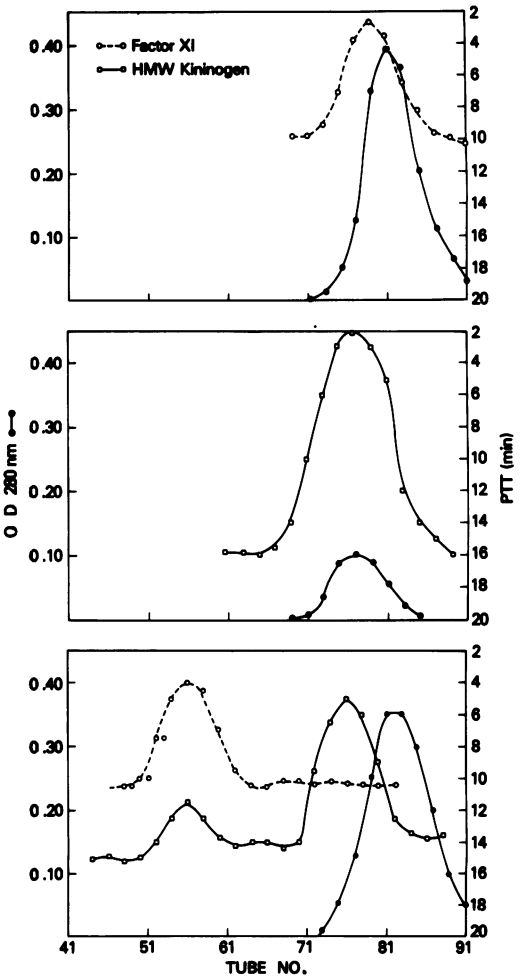


FIGURE 4 Sephadex G-200 gel filtration of 2 ml of Factor XI (5 U/ml) (upper panel), 2 ml of purified HMW kininogen (50 U/ml) (center panel), and a mixture of 2 ml Factor XI plus 2 ml HMW kininogen (bottom panel). Buffer was added so that a total of 4.0 ml was applied to the column in each case.

kininogen, and if a fraction thereof were complexed, the inhibitors present at mol wt 200,000–300,000 might have inhibited any plasmin generated in our assay. We therefore assayed the column for plasminogen by Ouchterlony analysis and a peak of antigenic plasminogen was found corresponding to the functional activity although none was found in the region of the prekallikrein-HMW kininogen complex. Furthermore, when fractions 55–70 were pooled and concentrated 10-fold, the concentrate had no detectable antigenic plasminogen.

To confirm the ability of Factor XI to bind to HMW kininogen, the Sephadex G-200 gel filtration was performed with highly purified components. Purified Factor XI alone was found to contain a small fraction of aggregated material. We therefore utilized Factor XI after the Sephadex G-150 gel filtration step before removal of IgG by immunoadsorption. As shown in the upper panel of Fig. 4, Factor XI coagulant activity was found at mol wt 175,000 just preceding the protein peak at OD 280 nm representing IgG. The center panel shows the chromatogram of the purified HMW kininogen. The protein peak and the functional activity were found at mol wt 210,000. The lower panel shows the fractionation of a mixture of the Factor XI with the HMW kininogen. The HMW kininogen was at a 10-fold excess by weight to approximate their relative concentrations in normal plasma. Utilizing the IgG as an internal marker it can be seen that the position of the OD 280 nm in the upper and lower panels show close correspondence. All of the Factor XI and ≅12% of the HMW kininogen was found at mol wt 380,000 whereas the excess HMW kininogen was again found at mol wt 210,000.

## DISCUSSION

The observations that the activation of Factor XI and prekallikrein by activated Hageman factor are both augmented by HMW kininogen and that Factor XI and prekallikrein-circulate bound to HMW kininogen suggests that these complexes are absorbed to negatively changed surfaces and that such binding facilitates the interaction of Hageman factor and its substrates. Because the concentration of HMW kininogen is ≅80 μg/ml whereas prekallikrein is 25 μg/ml, and Factor XI is 7 μg/ml (11), there is ample HMW kininogen present to completely bind both molecules. However the Factor XI-HMW kininogen complex is separable from the prekallikrein-HMW kininogen complex; thus, prekallikrein and Factor XI appear to bind to different molecules of HMW kininogen. It is possible that prekallikrein and Factor XI compete for the same binding site and competitive studies of their binding to HMW kininogen are in progress.

Because kallikrein, Factor XIa, and plasmin can each activate Hageman factor, we considered the possibility that plasminogen is also bound to HMW kininogen; however, no binding was evident. We have recently reported that Factor XIa-HMW kininogen activates surface-bound Hageman factor and that the HMW kininogen augments the subsequent activation of prekallikrein. However, no such augmentation by HMW kininogen was evident when plasmin was used to activate the Hageman factor. It therefore appears possible that Factor XIa-HMW kininogen may represent an alternative mechanism for the enzymatic activation of Hageman factor and contribute to the gradual activation observed upon exposure of prekallikrein-deficient plasma to negatively charged surfaces.

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