The Binding of Bovine Factor XII to Kaolin

By Edward P. Kirby and Patrick J. McDevitt

Purified bovine factor XII was radiolabeled with iodine-125 and its binding to kaolin studied. Binding was rapid and was not readily reversible upon adding unlabeled factor XII. The optimum pH for binding was in the region of pH 5-7. The isoelectric point of factor XII was pH 5.7. High concentrations of urea or increasing the ionic strength of the medium did not inhibit binding. Polyelectrolyte macromolecules, such as Polybrene and polylysine, were effective inhibitors of factor XII binding to kaolin. Polylysine caused the release of factor XII that had bound to the kaolin surface.

Physical and chemical studies of its interaction with activators.

MATERIALS AND METHODS

Materials

Aprotinin, bovine serum albumin, cytochrome-c, dextran sulfate (500,000 mol wt), dibothrioethitol, heparin (porcine intestinal mucosa), morpholinooethylsulfonic acid, myoglobin (sperm whale), polylysines (8000, 85,000, and 195,000 mol wt), trypsin, and wheat germ lectin were from Sigma Chemical Co., St. Louis, Mo. Acid-washed kaolin, sodium dodecylsulfate (SDS), and most general laboratory reagents were from Fisher Scientific, King of Prussia, Pa. Polybrene and dextran sulfate (DSPF) were from Aldrich, Milwaukee, Wis. Na<sup>125</sup>I (17 Ci/mg) and Na<sup>99</sup>Mo (25 Ci/mg) were from New England Nuclear, Boston, Mass. Acrylamide (electrophoresis grade) and ethyl (dimethylaminopropyl) carbodiimide were from BioRad Laboratories, Richmond, Calif.

Factor XII-deficient human plasma was obtained from George King Biomedical, Overland Park, Kans. Diethylaminoethyl (DEAE) Sephadex A-50, Sepharose 4B-CL, cyanogen bromide activated Sepharose 4B-CL, and wheat germ lectin Sepharose were obtained from Sigma. Carbamoyl methyl cellulose CM-52 was from Whatman, Ltd., Maidstone, England.

Buffers

PS buffer contained 0.01 M sodium phosphate, 0.15 M NaCl, 1.5 mM sodium azide, pH 6.5.

PSBSA buffer contained 0.01 M sodium phosphate, 0.15 M NaCl, 1.5 mM sodium azide, pH 6.5, plus bovine serum albumin (1 mg/ml).

Preparation of Corn Trypsin Inhibitor (CTI)

The trypsin inhibitor from corn, which specifically inhibits factor XII, was prepared by a modification of the method of Hojima et al.<sup>5</sup> All steps were performed at 4°C. Fresh sweet corn (3 kg) was extracted twice with 1200 ml of cold 0.2 M NaCl using a Waring blender and was filtered through cheesecloth. The filtrates were combined (5.7 liter) and centrifuged at 7000 g for 20 min. The supernatant (5.6 liter) was titrated to pH 4.0 with 130 ml of 1 M HC1 and centrifuged at 7000 g for 15 min. The pH was readjusted to 6.4 with 80 ml of 0.1 M NaOH, and the extract again centrifuged at 7000 g for 15 min. Solid ammonium sulfate was added to the supernatant to 60% saturation and stirred for 30 min. After centrifugation for 10 min at 7000 g, the precipitate was dissolved in 0.1 M Tris·0.15 M NaCl, pH 8.0, to a volume of 630 ml. The dissolved precipitate was dialyzed against 12 liters of deionized H2O, and then against 15 liters of 0.1 M Tris·0.15 M NaCl, pH 8.0. Following centrifugation at 7000 g for 20 min, 2 volumes (1260 ml) of cold acetone were slowly added with continuous stirring. The suspension was centrifuged at 7000 g for 20 min. The corn trypsin inhibitor was precipitated from the supernatant by adding an additional 2520 ml acetone, stirring for 60 min, and then centrifuging at 7000 g for 20

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min. The precipitate was dissolved in 0.02 M Tris-0.03 M NaCl buffer, pH 8.2, to a volume of 225 ml, and dialyzed against 4 liter of the same buffer. After centrifugation at 7500 g for 20 min, the CII was stored frozen at -90°C. CII was assayed by its ability to inhibit the amidolytic activity of purified trypsin. Electrophoresis of this preparation on reduced SDS polyacrylamide gels showed a single component with an apparent molecular weight of 14,000 daltons.

**Insolubilization of Proteins**

Aprotinin, wheat germ lectin, and corn trypsin inhibitor were insolubilized on cyanogen bromide-activated agarose obtained from Sigma Chemical Co. or prepared by the technique of March et al..

Unbound reactive sites on the activated agarose were blocked by incubation with 0.2 M ethanolamine, pH 9.5, for at least 4 hr, followed by extensive washing of the gel with buffer. Arginine-agarose and heparin-agarose were prepared as described by Fujikawa et al.

**Preparation of Bovine Factor XII**

Factor XII was isolated from bovine plasma by a modification of the method of Fujikawa et al. Bovine blood was collected into one-twentieth volume of 0.1 M sodium oxalate buffer, pH 7.0, containing benzamidine (64 mM), heparin (100 mg/liter), and crude soybean trypsin inhibitor (100 mg/liter). Plasma was obtained by centrifugation at room temperature through a Westphalia model SA00H-205 continuous flow centrifuge (Centrico, Inc., Northvale, N.J.) and then filtered through a Westphalia model SA00H-205 continuous flow centrifuge (Centrico, Inc., Northvale, N.J.) and then absorbed with barium sulfate (40 g/liter) for 20 min in the cold. After centrifugation (2500 g for 10 min), the plasma was frozen (-20°C) in 3-liter batches until needed.

Frozen plasma (10-12 liter) was partially thawed at room temperature overnight, then adjusted to pH 6.3 with a saturated solution of KH₂PO₄. All subsequent steps were performed in a cold room (4-8°C). Cold ethanol (-90°C) was added to a final concentration of 15%, and the resulting precipitate was removed by centrifugation. The supernatant was treated with ammonium sulfate (351 g/liter) and stirred for 30 min in the cold. Following centrifugation (7000 g for 15 min), the precipitate was resuspended in 500 ml cold deionized water containing soybean trypsin inhibitor (22 mg/liter), Polybrene (100 mg/liter), and DFP (1 mM). The resuspended precipitate (2.7 liter) was dialyzed against 32 liter deionized water, and then against 32 liter 0.03 M Tris-0.05 M NaCl buffer, pH 7.7. Polybrene (100 mg/liter) and DFP (3 x 10⁻⁵ M) were present during dialysis.

After centrifugation, the supernatant was adsorbed sequentially with 2 batches of DEAE Sephadex (2 liter each), which had been equilibrated with 0.03 M Tris-0.05 M NaCl buffer, pH 7.7. The second batch of adsorbed DEAE Sephadex was packed into a column (10 cm diameter), and then the first batch of adsorbed DEAE Sephadex was packed on top of it. A nonlinear salt gradient composed of 1.5 liter of 0.03 M Tris-0.05 M NaCl, pH 7.7, a mixing flask, and 4.0 liter of 0.05 M Tris-0.05 M NaCl, pH 7.7, was applied at a rate of 270 ml/hr. Fractions (75 ml) were collected from tubes containing 0.1 ml of a mixture of soybean trypsin inhibitor (10 mg/ml) and Polybrene (10 mg/ml). Fractions with factor XII activity were pooled (740 ml), treated with Polybrene (100 mg/liter) and DFP (7 x 10⁻⁴ M), and dialyzed against 16 liter of 0.05 M imidazole-0.06 M NaCl buffer, pH 6.0, containing DFP (3 x 10⁻⁵ M) and Polybrene (100 mg/liter).

The dialyzed pool was centrifuged and the supernatant was applied to a 6.5 x 13 cm column of heparin agarose in the same buffer at a rate of 400 ml/hr. The column was washed with 0.05 M imidazole-0.25 M NaCl, pH 6.0, and then the factor XII was eluted with 0.05 M imidazole-0.7 M NaCl, pH 6.0 at a rate of 480 ml/hr. Fractions containing factor XII were pooled (180 ml) and treated with soybean trypsin inhibitor (100 mg/liter), DFP (3 mM), and Polybrene (50 mg/liter). The pool was dialyzed against 16 liter of 0.5 M sodium acetate-0.06 M NaCl, pH 5.6, containing DFP (3 x 10⁻³ M) and Polybrene (50 mg/liter), then centrifuged and applied to a column (2.5 x 24 cm) of CM-cellulose equilibrated in the dialysis buffer. The column was washed with 250 ml of the same buffer at a flow rate of 2 ml/min. Factor XII was eluted from the column using a linear gradient composed of 250 ml each of 0.05 M NaAc-0.06 M NaCl, pH 5.6, and 0.05 M NaAc-0.6 M NaCl, pH 5.6. Each buffer contained Polybrene (50 mg/liter).

The pool from the CM-cellulose column was centrifuged and the supernatant applied to a column (1.5 x 16 cm) of arginine agarose equilibrated in the dialysis buffer (flow rate 40 ml/hr). The column was washed with 100 ml of this buffer, and factor XII was eluted with a linear gradient of 50 ml each of 0.02 M imidazole-0.02 M NaCl, pH 6.0, and 0.02 M imidazole-0.2 M NaCl, pH 6.0. Polybrene (50 mg/liter) was present in these buffers. Variable amounts of activated factor XII could subsequently be eluted from the column with 0.02 M imidazole-1 M NaCl, pH 6.0.

The factor XII pool (50 ml) was applied to a column (5 ml) of wheat germ lectin-Sepharose equilibrated in 0.02 M imidazole-0.02 M NaCl, pH 6.0, containing Polybrene (50 mg/liter). A small amount of equilibration buffer was used to wash the column, and factor XII was then eluted with equilibration buffer containing 1% N-acetyl-D-glucosamine at a flow rate of 2 ml/hr.

After dialysis, traces of activated factor XII in the preparation were removed by affinity chromatography on corn trypsin inhibitor-Sepharose. Small columns (1.5 ml) were prepared in siliconized Pasteur pipettes and the factor XII solution was applied at 5 ml/hr. Nonactivated factor XII eluted in the run-through fractions; activated factor XII could be subsequently eluted with 1 M acetic acid. Traces of any activating proteases, such as kallikrein, in the preparation (indicated by a slow activation of the factor XII during storage) were removed by chromatography on aprotinin-Sepharose, which binds kallikrein and plasmin, but not factor XII or XIIIa.

Purified factor XII was stored frozen at -90°C or could be kept at 4°C for greater than 1 mo without loss of activity.

**Assays**

Factor XII procoagulant activity was measured by a modification of the one-stage kaolin-activated partial thromboplastin time. Dilutions of the factor XII samples (0.1 ml) were mixed with 0.1 ml of factor-XII-deficient plasma (which had been diluted 1:2 with 0.01 M Tris-0.15 M NaCl, pH 7.4), and 0.1 ml of a mixture of phospholipid (0.03% Insolubin, Associated Concentrates, Woodside, N.Y.) and 0.25% kaolin in Tris-Cl buffer. After incubation for 5 min at 37°C, 0.1 ml of 0.025 M CaCl₂ was added and the clotting time measured with a Fibrometer coagulation timer (BBL, Cockeysville, Md.).

Amidolytic activity was assayed using 0.12 mM S-2302 (B-Pro-Phe-Arg-p-Nitroanilide, Ortho Diagnostics, Raritan, N.J.) in 0.1 M Tris buffer, pH 8.0. The change in absorbance at 405 nm was measured during incubation at 37°C. S-2302 is a good substrate for both XIa and kallikrein. Activity due specifically to factor XIa or to kallikrein was determined by preincubating the enzyme solution with either aprotinin or cryoprecipitate inhibitor to inactivate contaminant kallikrein or factor XIIa, respectively. Factor XIIa was activated by incubation with 0.02 U/ml of kallikrein in the presence of dextran sulfate (85 μg/ml). The partially purified kallikrein used had been obtained during the purification of prekallikrein by the method of Heimark and Davie and contained 2 amidolytic units/mg. One unit
of amidolytic activity is defined as the amount of enzyme causing the cleavage of 1 amole of peptide/min under our assay conditions.

**Polyacrylamide Gels**

Samples for electrophoresis were diluted with an equal volume of phosphate buffer containing 10% sodium dodecyl sulfate, 6 M urea, and, in some cases, 1% diisopropylthreitol. The samples were heated at 100°C for 3 min and applied to 7.5% polyacrylamide gels using the Weber-Osborn system. The gels were electrophoresed at 6 mA/gel for approximately 16 hr and then stained with 0.05% Coomassie brilliant blue R250 in 25% isopropanol, 10% acetic acid for 24 hr. They were destained in 10% acetic, 30% methanol, and then stored in 7% acetic acid. Gels were sliced using a multwire slicer (MRA Corporation, Clearwater, Fla.), and 2-mm segments were counted for radioactivity.

**Labeling of Proteins**

Purified single-chain factor XII was labeled using the iodine monochloride method. Na{sup 125}I (400 μCi) was added to 2 ml factor XII (0.5 mg/ml in 0.1 M phosphate buffer, pH 7.0). This solution was then incubated with 5 μL IC1 (3 mM in 0.01 M HCl, 0.1 M NaCl) for 2 min. Sodium metabisulfite was added to a final concentration of 1.5 × 10{sup -4} M to stop the reaction. Bovine serum albumin was then added to a final concentration of 1 mg/ml and the mixture was applied to a 10-ml column of Sephadex G-25 equilibrated in 0.1 M phosphate buffer, pH 7.0, containing BSA (1 mg/ml). Factor XII was eluted from the column using the same buffer. The radioactive fractions appearing in the void volume were pooled and then dialyzed extensively in the cold against PS buffer. The extent of labeling achieved by this procedure was approximately 0.4 molecules of iodine per molecule of factor XII, with little or no loss of procoagulant activity.

Bovine serum albumin was labeled with {sup 125}I using a similar procedure to obtain approximately 0.2 molecules of bound iodine per molecule of albumin.

**General Procedure for Binding Studies**

Fifty μl of radiolabeled factor XII (XII*) in PS buffer was added to a 0.6-ml polypropylene Eppendorf centrifuge tube containing 200 μl PSBSA buffer or the indicated reagent dissolved in PSBSA. Binding was initiated by the addition of 250 μl of kaolin suspension (in PSBSA). The samples were incubated at room temperature with continuous gentle inversion. The incubation was terminated by centrifugation for 10 sec in an Eppendorf centrifuge. The supernatant was removed with a Pasteur pipette that had been drawn out to a fine tip and siliconized (Dri Film SC-87, Pierce Chemicals, Rockford, Ill.). The pellet was obtained by cutting off the bottom 2 mm of the tube. Samples were counted in an Intertechnique Gamma Counter (model CG-4000). Binding efficiency was expressed as the percent of the total counts (pellet plus supernatant) that were found in the pellet. Under these conditions, no significant binding of factor XII* to the polypropylene tubes was observed. Unless otherwise noted, all concentrations listed are final concentrations.

**Isoelectric Focusing**

Isoelectric focusing was performed in a 110-ml column (Model 8100-1, LKB Instruments Inc., Rockville, Md.) using a 0.5%-47% sucrose gradient as described by Vesterberg. The low density solution contained 0.33% ampholines (by dry weight), and the high density solution contained 1.0%. The ampholines used were a 1:1 mixture of pH 3.5-10 and pH 4.0-6.0 Ampholytes (LKB Inst. Co.).

**RESULTS**

**Purification and Characterization of Bovine Factor XII**

Factor XII was purified by a modification of the procedure of Fujikawa et al. Each of the early steps in the preparation was conducted in the presence of Polybrene, to inhibit contact activation, as well as diisopropylfluorophosphate (DFP) and soybean trypsin inhibitor to block any serine proteases that might be present. Traces of activated factor XII or kallikrein in the final preparation were removed by affinity chromatography on insolubilized corn trypsin inhibitor or aprotinin, respectively. Corn trypsin inhibitor specifically binds factor XII*, but not factor XII or kallikrein. Aprotinin binds kallikrein, but does not bind factor XII or XII*. The purified factor XII was stable for greater than 1 mo when stored at 4°C and did not show any tendency to activate spontaneously during storage.

Bovine factor XII, as purified by our procedure, showed a single band after electrophoresis on both reduced and nonreduced SDS polyacrylamide gels (Fig. 1, A and B). The apparent molecular weight determined from reduced gels was 78,000 in good agreement with the value reported by Fujikawa et al. The protein was labeled with {sup 125}I by the iodine monochloride method. Incorporation of up to 2 iodine atoms per molecule of factor XII resulted in less than a 20% loss of procoagulant activity. Upon treatment with kallikrein and dextran sulfate, all of the labeled factor XII could be cleaved, yielding chain fragments similar in molecular weight to those described by Fujikawa et al. Amidase activity against S-2302 appeared in parallel with the first cleavage in the factor XII. Subsequent cleavage did not seem to be associated with any change in the amidase activity of the factor XII. No change in mobility of factor XII on nonreduced SDS-polyacrylamide gels was observed during the course of activation, suggesting that all of the fragments were held together by disulfide bonds.

**Binding of Factor XII to Kaolin**

Incubation of labeled factor XII with kaolin led to the binding of XII* to the kaolin surface (Fig. 2). At very high levels of kaolin, over 90% of the radioactive
factor XII could be bound. All binding studies were done in the presence of bovine serum albumin (1 mg/ml) to inhibit nonspecific binding of the labeled protein in the plastic tubes and to the kaolin. Higher concentrations of albumin caused only a slight further decrease in XII* binding.

In the presence of albumin (1 mg/ml), most of the XII* binding that was observed was specific, since it could be inhibited by high concentrations of unlabeled factor XII (Fig. 3A). The capacity of the kaolin was so high, however, that even at the highest concentrations of factor XII that we tested (greater than 50 μg/ml), an appreciable fraction of the XII* was bound. Similar
binding patterns were observed, whether binding was examined using varying concentrations of labeled factor XII or using a small concentration of labeled factor XII diluted with varying concentrations of unlabeled factor XII. This suggests that labeled and unlabeled factor XII were essentially identical in their binding affinities.

Binding of factor XII to kaolin was saturable, but was not a simple process. The binding was not completely reversible and Scatchard plots\(^{19}\) of binding isotherms were nonlinear. Graphing the binding data on double-reciprocal plots indicated that, at saturation, approximately 18 \(\mu\)g of factor XII could be bound per milligram of kaolin.

The binding of XII\(^*\) to kaolin was quite rapid (Fig. 4) and was essentially complete within 10 min. The binding of XII\(^*\) was substantially decreased by the prior addition of a large amount of unlabeled factor XII, but addition of unlabeled XII after binding had proceeded for 0.5 or 10 min did not lead to reversal of XII\(^*\) binding. No further binding occurred, but the majority of the XII\(^*\) that had already bound was apparently bound irreversibly. The rate of binding was substantially decreased at lower temperatures, but the total amount of XII\(^*\) bound after 20–30 min was approximately the same at all temperatures tested, in the range of 0–38\(^\circ\)C. No cleavage of factor XII occurred under the conditions of these incubations.

Fig. 4. Time course and nonreversibility of binding. \(^{125}\)I-labeled factor XII (0.85 \(\mu\)g/ml) was incubated with kaolin (300 \(\mu\)g/ml) at room temperature for increasing periods of time before centrifugation (O--O). To some samples, an excess of unlabeled factor XII (43 \(\mu\)g/ml) was added either initially (O--O), after 0.5 min of incubation (A--A), or after 10 min of incubation (V--V).

Fig. 5. Effects of pH on Factor XII binding. \(^{125}\)I-labeled factor XII (3.1 \(\mu\)g/ml) was added to samples of either 0.05 M citric acid, 0.1 M NaCl buffer (O--O), or 0.05 M sodium phosphate, 0.1 M NaCl buffer (A--A), which had been adjusted to various pH values with 2 N NaOH. Bovine serum albumin (1.0 mg/ml) and kaolin (300 \(\mu\)g/mn final concentration) were added and the samples incubated for 5 min at room temperature. The factor XII\(^*\) bound was determined after centrifugation. The final pH of each sample was measured after completion of the incubation.

Fig. 6. Isoelectric focusing of factor XII. \(^{125}\)I-labeled factor XII was mixed with myoglobin and \(^{131}\)I-labeled bovine serum albumin and subjected to isoelectric focusing for 20 hr at 800–1800 volts. After fractionation, samples were tested for \(^{125}\)I and \(^{131}\)I content in a gamma counter, the pH of the samples were determined, and myoglobin was detected by its absorbance at 421 nm.
Mechanisms of Factor XII Binding to Kaolin

Kaolin particles have a high surface negative charge so that binding of protein molecules, such as factor XII, might occur by simple, nonspecific electrostatic interactions. Optimal binding of factor XII to kaolin occurred in the region of pH 5.5–7, and was reasonably independent of pH in this region (Fig. 5). However, binding decreased sharply below pH 5. Isoelectric focusing of factor XII* revealed a peak of radioactivity between pH 5 and 6, with a maximum at pH 5.7 (Fig. 6). An isoelectric point of pH 5.7 suggests that at neutral pH, factor XII carries a net negative charge and so cannot be binding to kaolin due to a generalized electrostatic attraction. Regions of localized positive charge on factor XII may exist, and these regions might thus function as specific binding sites.

Increasing the ionic strength of the buffer to as high as 1.2 M, which would be expected to interfere with generalized electrostatic interactions, had little effect on the binding of factor XII to kaolin. Highly charged macromolecules, however, were very efficient inhibitors of factor XII binding (Table 1). These may compete either by binding to the factor XII or the kaolin. Dextran sulfate binds to factor XII and promotes its activation by kallikrein.1 Concentrations of dextran sulfate, which gave optimal activation of factor XII (i.e., <50 μg/ml), caused only partial inhibition of the binding of XII* to kaolin. Positively charged macromolecules, such as Polybrene, cytochrome-c, and polylysine, bind to kaolin and may compete with factor XII for negatively charged sites on the kaolin. Three forms of polylysine, with molecular weights of 8000, 85,000, and 185,000 were tested. All gave approximately the same inhibitory activity, when expressed on a per microgram basis. Surprisingly, polylysine was able to elute labeled factor XII from kaolin (Fig. 7), in contrast to the inability of unlabeled factor XII to reverse binding (Fig. 4).

Hydrogen bonding does not appear to play a major role in binding factor XII to kaolin, since high concentrations of urea, which disrupts hydrogen bonds, did not significantly inhibit binding (Table 2). Hydrophobic interactions, however, may play a role, since sodium thiocyanate, which is a very strong chaotropic agent,20 was a better inhibitor than equivalent concentrations of other salts, such as Na₂SO₄ (Table 2). The general order of effectiveness of the salts tested was NaSCN > NaI > NaCl, NaH₂PO₄ > Na₂SO₄.

DISCUSSION

The factor XII that we have purified from bovine plasma is very similar in its properties to that purified by Fujikawa et al.11 Labeling with iodine-125 by the iodine monochloride method did not significantly decrease the ability of the molecule to correct the coagulation defect in factor-XII-deficient plasma, and the molecule could be completely cleaved by kallikrein, yielding products that appeared identical to those obtained from unlabeled factor XII. Labeled and unlabeled factor XII appeared to compete on an equal

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Table 1. Effect of Polyvalent Ions on XII* Binding

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Alkaline and neutral pH inhibition of factor XII binding occurred in the region of pH 5.5–7, so that binding of protein molecules, such as factor XII, might occur by simple, nonspecific electrostatic interactions. Optimal binding of factor XII to kaolin was independent of pH in this region (Fig. 5). However, binding decreased sharply below pH 5. Isoelectric focusing of factor XII revealed a peak of radioactivity between pH 5 and 6, with a maximum at pH 5.7 (Fig. 6). An isoelectric point of pH 5.7 suggests that at neutral pH, factor XII carries a net negative charge and so cannot be binding to kaolin due to a generalized electrostatic attraction. Regions of localized positive charge on factor XII may exist, and these regions might thus function as specific binding sites.

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Fig. 7. Reversal of binding by polylysine. 1²⁵I-labeled factor XII (0.55 μg/ml) was incubated with kaolin (300 μg/ml) for varying periods of time before centrifugation to determine the extent of XII* binding (○—○). Polylysine (85,000 mol wt, 90 μg/ml) was added to samples either initially (□—□), after 0.5 min (△—△), or after 10 min (▽—▽) of incubation.
Table 2. Effect of Dissociating Reagents on XII* Binding

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<td>2.10</td>
<td>2.10</td>
<td>14.6</td>
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Factor XII* (6 µg/ml) was added to PSBSA buffer containing increasing amounts of urea, sodium sulfate, or sodium thiocyanate (adjusted so that their contributions to the final ionic strengths of the solutions were equivalent). Kaolin (200 µg/ml) was added, and the amount of XII* bound was determined by centrifugation after incubation at room temperature for 5 min.

basis for binding to kaolin, suggesting that the binding of labeled factor XII to kaolin is a suitable model for the study of the interaction between factor XII and a kaolin surface.

Our data suggest that there are specific interactions between regions of the factor XII molecule and specific regions of the kaolin surface, but that these interactions are rather complex. Excess unlabeled factor XII decreases binding of labeled factor XII to kaolin, but because of the high capacity of the kaolin for factor XII, saturation is difficult to achieve. Extrapolation from values at high factor XII concentrations (greater than 50 µg/ml) suggest that, at saturation, approximately 18 µg of factor XII can be bound per milligram of kaolin. This is, of course, probably very dependent on the particle size and surface area of the kaolin and would probably depend on the source of the kaolin, so that estimation of actual number of factor XII molecules bound per particle seems unwarranted. The lack of reversibility upon addition of excess unlabeled factor XII and the heterogeneity of the kaolin precludes analysis by the method of Scatchard. We can, however, make some conclusions about the mechanism by which factor XII binds to kaolin.

The lack of inhibition by increased ionic strength suggests that generalized electrostatic interactions do not play a major role in causing factor XII binding. Our observation that the isoelectric point of bovine factor XII is at pH 5.7 also diminishes the likelihood that binding is through generalized electrostatic interactions, since factor XII would carry a net negative charge at neutral pH. This pI is somewhat lower than the pI of 6.1–6.5 reported by Kaplan et al. for human factor XII, but in each case, the molecule would be negatively charged at pH 7.4. It may, however, have regions of localized positive charge responsible for its binding to kaolin.

The inability of excess unlabeled factor XII to reverse the binding of factor XII* to kaolin suggests that there may be a multiseite attachment of factor XII to kaolin or that bound factor XII* may undergo a conformational change leading to irreversible binding. This latter possibility would be supported by the observations of Griffin, who saw that binding of factor XII to activating surfaces increased its susceptibility to cleavage by kallikrein, and by the reports of McMillin et al. and Fair et al. who used circular dichroism and fluorescence techniques to observe conformational changes in factor XII when it bound to an activator.

Polylysine can bind to kaolin and probably inhibits factor XII binding by coating the kaolin particles. It can rapidly reverse factor XII* binding, perhaps by being able to interact at each of the multiple sites of factor XII* attachment, allowing several molecules of polylysine to simultaneously "pry loose" a factor XII* molecule, whereas unlabeled factor XII molecules cannot compete for all binding sites simultaneously and so do not reverse factor XII* binding.

REFERENCES

5. Griffin JH, Cochrane CG: Mechanisms for the involvement of
The binding of bovine factor XII to kaolin

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