Human blood coagulation Factor XIa was reduced and alkylated under mild conditions. The mixture containing alkylated heavy and light chains was subjected to affinity chromatography on high M<sub>r</sub> kinogen-Sepharose. Alkylation experiments using [14C]iodoacetamide showed that a single disulfide bridge between the light and heavy chains was broken to release the light chain. The alkylated light chain (M<sub>r</sub> = 35,000) did not bind to high M<sub>r</sub> kinogen-Sepharose while the heavy chain (M<sub>r</sub> = 48,000), like Factors XI and Xla, bound with high affinity. The isolated light chain retained the specific amidolytic activity of native Factor XIa against the oligopeptide substrate, pyroGlu-Pro-Arg-p-nitroanilide. K<sub>a</sub> and k<sub>a</sub> values for this substrate were 0.06 M<sup>-1</sup>s<sup>-1</sup> for both Factor XIa and its light chain, and the amidolytic assay was not affected by CaCl<sub>2</sub>. However, in clotting assays using Factor XIa, an excellent correlation was observed and the light and heavy chains was broken to release the light chain which was only 1% as active as native Factor XIa.

Human coagulation Factor IX was purified and labeled with sodium [3H]borylhydride on its carbohydrate moieties. When this radiolabeled Factor IX was mixed with Factor XIa, an excellent correlation was observed between the appearance of Factor IXa clotting activity and tritiated activation peptide that was soluble in cold trichloroacetic acid. Factor XIa in the presence of 5 mM CaCl<sub>2</sub> activated [3H]Factor IX to an amidolytically inactive zymogen form. The reactions that lead to the activation of Factor XI are initiated upon exposure of blood to negatively charged surfaces. They involve the reciprocal proteolytic activation of Factor XII and prekallikrein on the negatively charged surface (3-7). Activated Factor XII then activates Factor XI by limited proteolysis (8, 9). In this reaction high M<sub>r</sub> kinogen serves as a nonenzymatic cofactor (10, 11). High M<sub>r</sub> kinogen is thought to be responsible for binding Factor XI to surfaces adjacent to surface-bound Factor XIIa, thereby facilitating the action of Factor XIa on Factor XI (10, 13). Stimulated platelets are also able to promote the activation of Factor XI (14-16) and possess high affinity receptors for Factor XI (17).

Human Factor XI has an apparent M<sub>r</sub> of 160,000 and consists of two very similar or identical polypeptide chains that are held together by one or more disulfide bonds (8, 9, 18, 19). During the activation of Factor XI by Factor XIIa or trypsin an internal peptide bond in each of the two chains is cleaved giving rise to a pair of disulfide-linked heavy and light chains with M<sub>r</sub> values of 48,000 and 35,000, respectively (8, 9, 20). Studies using diisopropylphosphorofluoridate or antithrombin III showed that each of these inhibitors bound to the light chain of Factor XIa in a stoichiometry of 2 mol of inhibitor/1 mol of enzyme (M<sub>r</sub> = 160,000), suggesting that each light chain of Factor XIa bears an active site (9).

Factor XIa is the activator of Factor IX in the intrinsic pathway of blood coagulation (1, 21). This reaction occurs in a calcium-dependent two-step mechanism. Initially, an internal peptide bond in Factor IXa is cleaved, giving rise to a two-chain disulfide-linked inactive intermediate. This intermediate is then converted to Factor IXa by a second cleavage due to Factor XIa, resulting in the release of an activation peptide (21, 22).

This paper describes studies of the functional roles of the...
heavy and light chain regions of Factor XIa. First, we isolated the alkylated light chain of Factor XIa using a method recently developed for this purpose for human plasma kallikrein (23). Then the isolated alkylated light chain was compared to native Factor XIa in terms of its activity on the oligopeptide substrate, pNA, its procoagulant activity, and its activity on 'H-Factor IX.

**MATERIALS AND METHODS**

All chemicals were the best grade commercially available.

**Purification of Proteins**—Factor XI and high M, kininogen were purified from human plasma using previously published methods (24). Factor XI was purified to greater than 95% homogeneity and contained 250 clotting units/mg of protein when the protein content was determined using the Lowry method (25) with a BSA standard. High M, kininogen was analyzed on SDS-polyacrylamide gels in the presence or absence of reducing agents and migrated as a single polypeptide chain of apparent M, = 110,000. Its specific coagulant activity was 14 clotting units/mg of protein.

Factor IX was purified from commercial Factor IX concentrate. Twelve bottles of Proplex (Hyland Therapeutics, Glendale, CA, Lot 958-C205) were taken up in 10 ml each of 0.05 M phosphate, pH 5.9, containing 40 mM benzamidine, 0.02% NaN, 10 mM EDTA, and 75 mM NaCl (final 18.5 mmho). These were pooled and dialyzed extensively in the same buffer and loaded on DEAE-Sephadex A-50 column (2.5 × 15 cm) at 30 ml/h. The resin was extensively washed to elute Factor VII. Factors IX and X and thrombinogen were eluted in a single peak with a salt gradient.

The dialyzed material was made 2.5 mM in CaCl₂ and loaded at 30 ml/h on a heparin-nagrose column (2.5 × 15 cm) equilibrated in the same buffer containing 2.5 mM CaCl₂. The column was extensively washed at 30 ml/h to elute Factor X. Factor IX was then eluted with a salt gradient using this same buffer containing 0.4 mM NaCl (39 mmho) as terminal buffer. Six-mL fractions were collected in tubes containing 100 μl of 1 M EDTA. The Factor IX was more than 95% pure as judged by SDS-polyacrylamide gels and contained 166 clotting units/mg.

β-Factor XIIa (M, = 28,000) was prepared by incubation of Factor XII with trypsin. β-Factor XIIa was then separated from trypsin by electrophoresis on a Beckman 121 A0 instrument (28). The reduced Factor XIa was alkylated by incubation at 37°C in the dark under nitrogen. The alkylation was performed using a mixture consisting of 0.77 μmol of [3H]iodoacetamide (Amer sham Corp., 54 mCi/mmol) and 0.07 μmol of unlabeled iodoacetamide. After dialysis against 0.165 M sodium acetate, 0.45 M benzamidine, pH 5.3, the light and heavy chains of Factor XIa were separated as described above. The concentration of light chain was determined based on its specific amodic activity as described elsewhere (Fig. 2). Samples were counted in a Beckman LS7500 β counter at Betaphase scintillation fluid, using a known amount of 0°C as standard.

**Amidolytic Assays of Factor XIa and Its Light Chain**—The kinetic parameters, Kₘ and kₗ, for the hydrolysis of pyroGlu-Pro-Arg-pNA by Factor XIa or its light chain were determined as follows. Ten μl of Factor XIa (228 μg/ml) or 10 μl of its light chain (0.70 μg/ml) were added to a 1-cm cuvette containing 490 μl of the substrate solution in 0.09 M Tris, 0.09 M NaCl, 0.005 M EDTA, pH 8.3. The initial rate of hydrolysis of duplicate samples was measured spectrophotometrically at 405 nm using a Cary 210 spectrophotometer. Kₘ and kₗ values were calculated from Lineweaver-Burk plots (30). Protein concentrations were determined using acid analysis.

**Clotting Assays of Factor XIa and Its Light Chain**—The procoagulant activity of Factor XIa or its light chain was determined using Factor XI-deficient plasma (George King Biomedical, Inc., Overland Park, Kansas). Fifty μl of deficient plasma was preincubated at 37°C for 30 s with 50 μl of Factor XIa or its light chain in different dilutions in 0.01 M Tris, 0.15 M NaCl, 1 mg/ml of BSA, pH 7.4 (TBS/BSA). Factor XI-deficient plasma (Sigma) (250 μl) or TBS/BSA were then added and further incubated at 37°C for 30 s before the mixture was recalcified with 50 μl of 0.05 M CaCl₂ and the clotting time measured. The observed clotting time was converted to clotting units by comparing the clotting activities of serial dilutions of a standard pool of normal human plasma as described before (8). A new clotting assay was performed for each coagulant species.

**Assay of 'H-Factor IX Activation**—The rate of release of the 'H-Factor IX activation peptide was followed by measurement of the trichloroacetic acid-soluble activation peptide as described before for bovine Factor X (31), bovine Factor IX (32), and human Factor X (33). Factor IX was tritiated and then subjected to preparative polyacrylamide electrophoresis as previously described (33). 'H-Factor IX contained 50,000 cpm/μg and retained full procoagulant activity.

For the studies reported in Figs. 3 and 4, 40 μl of 'H-Factor IX (4.3 clotting units/ml), 150 μl of TBS containing 10 mg/ml of BSA, and 24 μl of 0.5 M Tris-Cl, pH 7.4, were mixed at 37°C with 24 μl of different dilutions of Factor XIa or light chain in TBS containing 10 mg/ml of BSA. Then 24 μl of 0.09 M NaCl, 0.005 M EDTA, or 24 μl of TBS was immediately added, mixed, and incubated at 37°C. At various times, 80-μl aliquots were withdrawn and added to 240 μl containing 33 mM EDTA, 3.3 mM Tris, 50 mM NaCl, 3.3 mg/ml of BSA, pH 7.4, at 0°C. One hundred sixty μl of ice-cold 15% trichloroacetic acid was added, mixed for 2 min, and incubated for 25 min. Triplicate aliquots (130 μl) of the supernatant were added to 8 ml of Betaphase scintillation fluid (Westchem Products, San
RESULTS

Preparation and Isolation of Heavy and Light Chains of Factor XIa—Purified Factor XI was activated by β-Factor XIa as described under “Materials and Methods.” Factor XIa had an apparent M₉ of 160,000 (Fig. 1A). As seen on SDS gels under reducing conditions in Fig. 1B, the resulting Factor XIa consisted of heavy chains of M₉ = 48,000 and light chains of M₉ = 35,000. A small amount (<10%) of Factor XI was not activated and migrated on reduced SDS gels at M₉ = 80,000.

To prepare the heavy and light chains, 1.1 mg of Factor XIa was reduced by 0.084 mM dithiothreitol in the presence of 7 mM benzamidine at pH 8.3 under nitrogen in the dark. After 45 min at 37 °C, the reduced Factor XIa was alkylated with 0.19 mM iodoacetamide for 45 min. Gel analysis of Factor XIa following reduction and alkylation showed that the reaction mixture contained the heavy and light chains and polypeptides of M₉ = 130,000, 100,000, and 80,000, presumably representing partially reduced Factor XIa, as well as unactivated Factor XI (Fig. 1C). In contrast to the light chain, a faint band of the heavy chain (Fig. 1C) was observed when compared to completely reduced Factor XIa (Fig. 1B). Therefore, it seems likely that the polypeptide bands in Fig. 1C of approximate M₉ = 130,000 and 100,000 consist mostly of the heavy chain.

The reduced and alkylated Factor XIa was dialyzed and then applied to a column containing high M₉, kininogen-Sepharose. Subsequently, the column was washed and the bound material was eluted with 0.5 M NaCl. The material that did not adhere to the column was shown by SDS-polyacrylamide gel electrophoresis to consist mostly of the light chain and a small amount of polypeptides with higher molecular weight. This material was removed by a second passage over high M₉, kininogen-Sepharose. Then the nonadherent material showed a single band on reduced SDS gels of M₉ = 35,000 (Fig. 1D), representing the purified alkylated light chain. This material was used for subsequent studies of the enzymatic activity of the light chain of Factor XIa. The material that bound to high M₉, kininogen-Sepharose and was eluted with 0.5 M NaCl was analyzed on unreduced SDS gels. A band of M₉ = 48,000 corresponding to the heavy chain and bands probably representing the partially reduced Factor XIa was observed.

Incorporation of [³⁵S]Acetamide into the Light Chain of Factor XIa—[³⁵S]Acetamide—Factor XIa light chain was prepared as described under “Materials and Methods.” A total of 0.97 ± 0.09 mol of acetamide was incorporated per mol of light chain.

Amino Acid Composition of Factor XIa and Its Light Chain—The amino acid composition of Factor XIa and its light chain was determined and is shown in Table I. The amino acid composition of Factor XIa was not significantly different from the light chain.
different from the composition previously reported for human Factor XI (8).

Comparison of the Amidolytic Activities of Factor XIa and Its Light Chain—In order to determine whether the light chain had retained the activity of native Factor XIa, the ability of these two proteins to hydrolyze the small substrate pyroGlu-Pro-Arg-pNA was compared. Fig. 2 shows Lineweaver-Burk plots of the amidolytic activities of Factor XIa and its light chain against this oligopeptide substrate. The $K_m$ for pyroGlu-Pro-Arg-pNA obtained from this plot was 0.56 mM and the $K_{cat}$ was 350 s$^{-1}$. These data (Fig. 2) indicate that the light chain retained the full amidolytic activity of Factor XIa. The fact that the kinetic parameters for dimeric Factor XIa and the single chain light chain are the same suggests that at least for a small substrate the two active sites of dimeric Factor XIa act independently of one another with no cooperative interactions. The material that adhered to the high $M_r$ kininogen-Sepharose column and that was eluted by high salt consisted of heavy chain and higher $M_r$ forms. This material displayed very low amidolytic activity, probably due to small amounts of partially reduced Factor XIa, which had bound to high $M_r$ kininogen-Sepharose and coeluted with the alkylated heavy chain.

Factor IX, a natural substrate of Factor XIa, requires calcium ions for a maximal rate of activation by this enzyme. We, therefore, tested the effect of 5 mM CaCl$_2$ on the cleavage of the small substrate, pyroGlu-Pro-Arg-pNA, by Factor XIa or light chain. Table II shows that calcium ions had no significant effect on the activity of either of the two enzymes on this oligopeptide substrate.

Procoagulant Activities of Factor XIa and Its Light Chain—The ability of Factor XIa and its light chain to correct the clotting defect of Factor XI-deficient plasma was determined (Table III). In the presence of kaolin, Factor XIa was 100 times as procoagulant as its light chain, whereas in the absence of kaolin it was 340 times as procoagulant. The presence

![Graph showing Lineweaver-Burk plots](https://example.com/plot.png)

**Fig. 2.** Lineweaver-Burk plots of the amidolytic activity of Factor XIa and its light chain against the oligopeptide substrate, pyroGlu-Pro-Arg-pNA. Amidolytic activities were determined as described under "Materials and Methods." Factor XIa (C) and its light chain (O) were present at 28 nM and 19 nM, respectively, in assay mixtures. The line is a least squares fit of the means of duplicate determinations. The correlation coefficient was 0.999. Protein molar concentrations were calculated using $M_r$ values of 80,000 and 35,000 for Factor XIa and its light chain, respectively. The $M_r$ value of 80,000 represents the $M_r$ per active site of Factor XIa for purposes of comparison, since Factor XIa contains two light and two heavy chains per $M_r = 160,000$.

| Substrate | Factor XIa | Light chain |
|-----------|------------|-------------|
| $\mu$M    | CaCl$_2$   | Control     | CaCl$_2$ | Control     |
| 92        | 51.5       | 51.3        | 54.0     | 55.2        |
| 184       | 91.8       | 89.3        | 89.8     | 86.9        |
| 368       | 151        | 147         | 140      | 149         |

*Amidolytic activity was determined as described in the legend to Figure 2. 5 mM CaCl$_2$ was present in the cuvette where indicated. The activity is expressed as micromoles of pNA released/μmol of enzyme/s.

![Graph showing TCA soluble activity](https://example.com/tca.png)

**Fig. 3.** Comparison of Factor XIa and its light chain in the activation of $^3$H-Factor IX. Factor XIa or its light chain at the indicated concentrations was incubated with $^3$H-Factor IX (3.8 μg/ml) in the presence of 5 mM CaCl$_2$ at 37°C as described under "Materials and Methods." Aliquots were removed at various times and the trichloroacetic acid (TCA)-soluble radioactivity was determined. Factor XIa was present at 0.26 nM (●), 0.084 nM (♦), or 0.028 nM (○). The light chain was present at 54 nM (■), 18 nM (▲), or 2.7 nM (●).
of kaolin had no effect on the ability of Factor XIa to correct the clotting time of Factor XI-deficient plasma, whereas the presence of kaolin increased the activity of the light chain approximately 4-fold (Table III).

**Cleavage of the Activation Peptide from 3H-Factor IX by Factor XIa or Its Light Chain**—Due to the difference in specific clotting activities observed for Factor XIa or its light chain, the presence of calcium ions was of interest to determine whether this was caused by a differential ability of these two enzymes to activate Factor IX in studies with purified proteins. Therefore, human Factor IX was purified and titrated on its carbohydrate, and the titrated activation peptide release assay described by Nemerson and his colleagues (31, 32) was applied to human Factor IX.

Different amount of Factor XIa or light chain were added to an incubation mixture containing 3H-Factor IX and 5 mM CaCl₂. The release of the trichloroacetic acid-soluble tritium was determined as a function of time. Between 0.028 nM and 0.25 nM of Factor XIa, the initial rate of release of trichloroacetic acid-soluble tritium from 3H-Factor IX displayed a linear increase with the concentration of Factor XIa (Fig. 3). To examine the effect of calcium ions on the activity of Factor XIa or its light chain, the release of the 3H-Factor IX activation peptide was measured in the presence of 5 mM CaCl₂ or 2 mM EDTA or buffer (Fig. 4). The presence of calcium ions stimulated the activity of Factor XIa approximately 600-fold in this assay. However, the activity of the light chain was decreased by about two-thirds in the presence of calcium ions. The presence of EDTA in comparison to Factor XIa or its light chain, the release of the 3H-Factor IX activation peptide was determined as a function of time. Between 0.028 nM and 0.25 nM of Factor XIa, the initial rate of release of trichloroacetic acid-soluble tritium from 3H-Factor IX displayed a linear increase with the concentration of Factor XIa (Fig. 3). To examine the effect of calcium ions on the activity of Factor XIa or its light chain, the release of the 3H-Factor IX activation peptide was measured in the presence of 5 mM CaCl₂ or 2 mM EDTA or buffer (Fig. 4). The presence of calcium ions stimulated the activity of Factor XIa approximately 600-fold in this assay. However, the activity of the light chain was decreased by about two-thirds in the presence of calcium ions. The presence of EDTA in comparison to buffer alone had no significant effect on either the activity of Factor XIa or its light chain. When the activities of the two enzymes were compared in the presence of 2 mM EDTA or buffer alone, the light chain exhibited approximately 85% of the activity of Factor XIa on a molar basis (Fig. 4).

**FIG. 4.** Effect of calcium ions or EDTA on 3H-Factor IX activation by Factor XIa and its light chain. Factor XIa or its light chain was incubated with 3H-Factor IX as described under “Materials and Methods,” in the presence of 5 mM CaCl₂, 2 mM EDTA, or buffer alone. The light chain was assayed at 54 nM in the presence of buffer (■), EDTA (△), or CaCl₂ (○). Factor XIa was assayed at 27 nM in the presence of EDTA (□) or buffer (○). The presence of CaCl₂, Factor XIa was assayed at 0.028 nM (○). TCA, trichloroacetic acid.

**Table IV**

| Enzyme | Cephalin | High M, kininogen | Kaolin | Initial rate |
|--------|----------|------------------|--------|-------------|
| Factor XIa | - | - | - | 6.1 |
| - | + | - | - | 5.5 |
| - | - | + | - | 5.1 |
| - | - | - | + | 4.6 |
| Light chain | + | - | - | 0.0032 |
| - | + | - | - | 0.0034 |
| - | - | + | - | 0.0035 |
| - | - | - | + | 0.0040 |

Full procoagulant activity of Factor XI in plasma is not expressed unless high M₆, kininogen, a negatively charged surface, and phospholipids are present together. It is well known that the negatively charged surface and high M₆, kininogen participate in the reactions leading to the activation of Factor XI, but it is not known whether they also might participate in the activation of Factor IX by Factor XIa. Similarly it is possible that phospholipids also may contribute to the activation of Factor IX by Factor XIa. Therefore, we examined the effect of cephalin, high M₆, kininogen, and kaolin on the activity of Factor XIa or its light chain using the 3H-Factor IX activation peptide release assay. As seen in Table IV, the presence of cephalin or high M₆, kininogen had no enhancing effect in this assay for either the activity of Factor XIa or its light chain. Kaolin had a slight inhibitory effect on the activity of Factor XIa, either alone or in combination with high M₆, kininogen (Table IV). Higher concentrations of kaolin in the reaction mixture, such as 2 mg/ml, either alone or in combination with high M₆, kininogen inhibited the activity of Factor XIa by 96%.

**DISCUSSION**

This study was undertaken to examine relationships between the functional properties and physical domains of human Factor XIa. Limited proteolysis of Factor XI with β-Factor XIla results in its activation to form Factor XIa (8, 9). Human Factor XIa is composed of four disulfide-linked polypeptide chains, including two heavy chains (Mᵣ = 80,000) and two light chains (Mᵣ = 35,000) (8, 9, 20). Conditions are described here under which mild reduction and alkylation of Factor XIa may be performed and a fully active light chain enzyme may be recovered. Fig. 1 shows that under the conditions of mild reduction not all of the Factor XIa molecules were completely reduced. Partially reduced forms of Factor XIa were present at Mᵣ = 130,000, 100,000, and 80,000. The band at Mᵣ = 80,000 probably represents reduced Factor XIa and/or two-chain Factor XIa. The band at Mᵣ = 100,000 might represent a dimer of heavy chains, suggesting that interchain disulfide bond(s) of Factor XIa are located between the heavy chain domains. The 130,000 Mᵣ band may represent
a complex consisting of two heavy chains and one light chain. The alkylated heavy and light chains of Factor XIa are separated using affinity chromatography on high M, kininogen-Sepharose using conditions similar to those employed for the isolation of Factor XI.² The light chain of Factor XIa like the light chain of kallikrein (23) does not adhere to high M, kininogen-Sepharose, whereas the heavy chain and other forms of Factor XI or Xla bind and are eluted with 0.5 M NaCl. Since Factor XI is known to circulate in plasma in a noncovalent complex with high M, kininogen (12), these results localize the high affinity binding site for high M, kininogen on the heavy chain of Factor Xla. Analogous studies have shown that the heavy chain region of kallikrein possesses the high affinity binding site for high M, kininogen (23).

The isolated alkylated light chain of Factor XIa retained full enzymatic activity against the artificial substrate, pyroGlu-Pro-Arg-pNA. For both Factor XIa and its light chain, the Kₐ and kₐ values for the hydrolysis of this substrate were, respectively, 0.56 mM and 350 s⁻¹. This indicates that the active site is located on the light chain and is not affected by the reduction and alkylation procedure. It also demonstrated that the heavy chain of Factor XIa is not important for the catalytic activity of the active site itself. Furthermore, no cooperative effects between the two equally potent active sites of dimeric Factor XIa are apparent. Studies have shown that both light chains of Factor XIa incorporate [³H]diproparglyphosphorofluoridate and react with antithrombin III (9). Hence, it was inferred that both light chains contain an active site. Moreover, an amino acid sequence typical of an active site serine region is located in the light chain (34).

Since Factor XIa and its light chain exhibit identical activities against a small substrate, we next examined the question of their relative activities in the more physiological setting of a clotting assay. The fact that Factor XIa is 100-fold more active than its light chain in a clotting assay (Table III) indicates that the heavy chain of Factor XIa has a very important role in coagulation reactions. Kaolin had no effect on the procoagulant activity of Factor XIa and it had a 4-fold stimulatory effect on that of the light chain.

One obvious explanation for the role of the heavy chain in expressing the procoagulant activity of Factor XIa in plasma would relate to the interaction of Factor XIa with its natural substrate, Factor IX. In order to explore this possibility, we studied the activation of purified Factor IX by Factor XIa or its light chain using the initiated Factor IX activation peptide release assay (31–33). In this assay Factor XIa proved to be approximately 2000 times as active as the light chain in the presence of CaCl₂. The two forms of the enzyme, however, exhibited the same activity against [³H]Factor IX in the absence of CaCl₂. Interestingly, calcium ions inhibited the activity of the light chain approximately 65%. Factor XIa in the presence of calcium ions was 600 times more active than the light chain or Factor XIa in the absence of calcium ions. These unexpected findings suggest that the heavy chain of Factor XIa is intimately involved in calcium-dependent mechanisms that accelerate the activation of Factor IX.

The role of calcium ions in the activation of Factor IX by Factor XIa has usually been assumed to be confined to interactions of calcium ions with Factor IX (35–37) since Factor IX binds calcium ions and no direct evidence has been published bearing on the interaction of calcium ions with Factor XI or Factor XIa. Human Factor IX binds 16 calcium ions and positive cooperativity was demonstrated for at least the first four sites (36, 37). A slight conformational change may occur upon calcium binding to Factor IX as evidenced by perturbations of tryptophanyl chromophores (37), although no large changes occur in the circular dichroism spectrum (36). Our data may suggest that the heavy chain of Factor XIa has a binding site for calcium ions which is required for a maximal rate of activation of Factor IX. Another interpretation is that calcium ions are required for Factor IX to be in a conformation that is necessary or optimal for interaction with the heavy chain of Factor XIa. Interaction of Factor IX in the presence of calcium ions with the heavy chain of Factor XIa could accelerate the reaction by enhancing enzyme-substrate interactions. Still there is no direct evidence that the heavy chain of Factor XIa interacts directly with Factor IX in the presence of calcium.

It is possible that differences in the activities exhibited by Factor XIa and its light chain are not due to functional properties provided by the heavy chain in the former, but rather due to a change in conformation of the light chain that occurs upon reduction and alkylation of intrachain disulfide bridges or upon alkylation of residues other than cysteine. These two possibilities are unlikely, since the experiment using [¹⁴C]iodoacetamide showed that only one molecule of acetamidethione is incorporated per molecule of Factor XIa light chain. This also shows that only one disulfide bridge attaches the light chain to the heavy chain and that the heavy chains are linked to each other by disulfide bonds.

No significance has yet been associated with the fact that Factor XIa is a covalent dimer containing two active sites. Disulfide bonds between the heavy chains of Factor XIa are essential for the dimeric nature of Factor XIa and the possibility of noncovalent interactions between the light chains cannot be excluded. Perhaps the dimeric nature of Factor XIa is important for efficiently cleaving two different peptide bonds in Factor IX, although with small substrates each active site of the dimeric Factor XIa is equally as active as the active site of the monomeric light chain.

The experiments shown in Table IV imply that the contributions of negatively charged surfaces and high M, kininogen to the intrinsic coagulation pathway are limited to the activation of Factor XI. Once Factor XIa is formed, kaolin and high M, kininogen have no influence upon its activity in the cleavage of purified [³H]-Factor IX. Similarly, phospholipids do not influence the activity of Factor XIa on Factor IX and hence their role is probably restricted to the intermediate and terminal stages of the coagulation pathways.

Factor XI and plasma prekallikrein are very similar in size, isoelectric point, and NH₂-terminal sequence (for review, see Refs. 1 and 39). They each bind reversibly to high M, kininogen, a cofactor in their surface-dependent activation by Factor XIIa. Moreover, Factor XIa as well as kallikrein are potent activators of surface-bound Factor XII and weak activators of plasminogen. Recently we reported studies of human plasma kallikrein (23) similar to those in this paper. As with Factor XIa, the light chain of kallikrein contains the active site and the heavy chain possesses the high affinity binding site for high M, kininogen. The procoagulant activities of the alkylated light chains are greatly decreased although amidolytic activities are fully retained. Unlike the light chain of Factor XIa, however, the light chain of kalli-krein retained full proteolytic activity against the macromolecular substrate, Factor XII, in solution. The large difference between the procoagulant activity of kallikrein and its light chain probably reflects the inability of the light chain of kallikrein to activate surface-bound Factor XII as effectively as does native kallikrein.³ The large difference between the procoagulant activity of Factor XIa and its light chain is due

³G. Tans and J. H. Griffin, unpublished observations.
to the calcium-dependent cleavage and activation of Factor IX. Thus, the heavy chain regions of Factor XIa and kallikrein not only contribute to the Factor XIIa-dependent activation of these molecules by binding to the surface cofactor, high-M, kininogen, but also contribute substantially to the action of these enzymes on their macromolecular substrates of the coagulation pathways.

Factor XIa activates Factor IX in the absence of calcium ions although at a much reduced rate as shown here and previously (36-38). Kallikrein also activates Factor IX but calcium ions do not accelerate this reaction and kallikrein is 20,000 times less active than Factor XIa (22). Measurements of the release of tritiated activation peptide from ³H-Factor IX by kallikrein and its isolated light chain showed that the light chain of kallikrein was as active as kallikrein in the presence or absence of calcium. Moreover, the light chain of Factor XIa was approximately 10 times as active as the light chain of kallikrein. Thus, the heavy chain of Factor XIa does not contribute to the ability of that enzyme to activate Factor IX and there is no calcium effect on the reaction. These observations reinforce the notion that the heavy chain region of Factor XIa somehow contributes critically to calcium-dependent mechanisms that enhance the activation of Factor IX. Since neither phospholipid nor kaolin enhance the activation of Factor IX by Factor XIa, calcium ions must directly enhance enzyme-substrate interactions.

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