Factor IX Activation by Factor XIa Proceeds without Release of a Free Intermediate†

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ABSTRACT: Factor IX activation by factor XIa is thought to proceed through the singly-cleaved free intermediate, factor IXα. However, we observed no intermediate development during factor IX activation by factor XIa when using a low substrate to enzyme ratio (44:1 mol/mol). This result can be explained by one of two mechanisms: (1) factor XIa-catalyzed activation proceeds via a singly-cleaved free intermediate with a much higher efficiency of cleavage than factor IX zymogen, or (2) the reaction occurs without free intermediate generation, whereby factor XIa makes both proteolytic cleavages in a single substrate molecule before releasing the final product (processive mechanism). We compared the factor XIa cleavage rates of free factor IXα and factor IXaα with that of factor IX zymogen. In contrast to the requirements of mechanism (1), the cleavage rate constants of factor IX zymogen, factor IXα, and factor IXaα were similar: 0.38 ± 0.02 s^{-1}, 0.34 ± 0.05 s^{-1}, and 0.27 ± 0.01 s^{-1}, respectively. It seems likely that factor XIa-generated intermediates observed under some reaction conditions are produced through the occasional failure of a processive mechanism. Indeed, in reactions using a high substrate to enzyme ratio (1900:1 mol/mol), we observed some factor IXα development; however, the pattern of intermediate and product development over time was inconsistent with a mechanism involving an obligate intermediate. Rather, it corresponded to behavior expected from a processive mechanism undergoing a consistent low failure. We conclude that factor XIa-catalyzed activation of factor IX proceeds via a processive mechanism without release of a free intermediate.

Factor IX is a 57 kDa zymogen glycoprotein which circulates in plasma at a concentration of approximately 90 nM (DiScipio *et al*., 1977; Osterud *et al*., 1978). Factor IX becomes active after cleavages between residues 145/146 and 180/181, which releases a 10 kDa glycosylated activation peptide, resulting in a two-chain, disulfide-linked molecule (Fujikawa *et al*., 1974; Lindquist *et al*., 1978) (Figure 1). Activated factor IX (factor IXaβ),[1] with its cofactor, factor VIIIa, then activates factor X in the tenase complex. Deficiencies in factor IX synthesis, activation, or activity result in the bleeding diathesis, hemophilia B, which ranges from mild to severe, depending on the plasma antigen concentration and level of remaining protein function (Giannelli *et al*., 1994).

Several proteins and protein complexes activate factor IX to factor IXaβ. Both the factor VIIa/tissue factor complex and factor XIa are physiological activators of factor IX in the extrinsic and intrinsic pathways, respectively (DiScipio *et al*., 1978; Lindquist *et al*., 1978; Osterud & Rapaport, 1977). Lawson and Mann (1991) showed that factor Xa is capable of cleaving factor IX between residues 145 and 146, producing the singly-cleaved species, factor IXα. A protease in Russell's Viper Venom cleaves factor IX between residues 180 and 181, converting factor IX to factor IXaα, which has approximately 20% of the activity of factor IXaβ (Lindquist *et al*., 1978). Monroe *et al*. (1985) showed that trypsin is able to catalyze factor IX activation.

The extrinsic pathway activator of factor IX, factor VIIa, is a 50 kDa serine protease which activates factor IX in the presence of calcium ions, a negatively charged phospholipid surface, and the factor VIIa cofactor, tissue factor (Lawson *et al*., 1992). Lawson and Mann (1991) characterized the activation of factor IX by factor VIIa/tissue factor and showed that activation by the extrinsic pathway complex proceeds via the factor IXα intermediate, which is subsequently converted to factor IXaβ.

The intrinsic pathway activator of factor IX, factor XIa, is a serine protease dimer consisting of identical disulfide-linked molecules. The role of the intrinsic pathway in hemostasis is unclear since individuals lacking the intrinsic pathway initiators (factor XII, prekallikrein, and high molecular weight kininogen) exhibit a normal coagulant response (Schmaier *et al*., 1988). However, deficiencies in factor XI cause mild to severe bleeding disorders which usually present only after significant trauma or in surgery (Rapaport *et al*., 1961; Sidi *et al*., 1978), suggesting a role for factor XIa in more demanding responses of hemostasis. Factor XIa does not require a membrane or protein cofactor for factor IX activation (DiScipio *et al*., 1978; Lindquist *et al*., 1978; Osterud *et al*., 1978); however, it has been shown

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[1] Abbreviations: factor IXα, factor IX cleaved between residues 145/146; factor IXaα, factor IX cleaved between residues 180/181; FXIa, factor XIa; RVV-X, Russell's Viper Venom-X; TBS, Tris-buffered saline (20 mM Tris, pH 7.3, 137 mM NaCl); TBS-T, Tris-buffered saline containing 0.1% Tween 20 (Bio-Rad); SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PC, phosphatidylcholine; PS, phosphatidylserine; TFPI, tissue factor pathway inhibitor.

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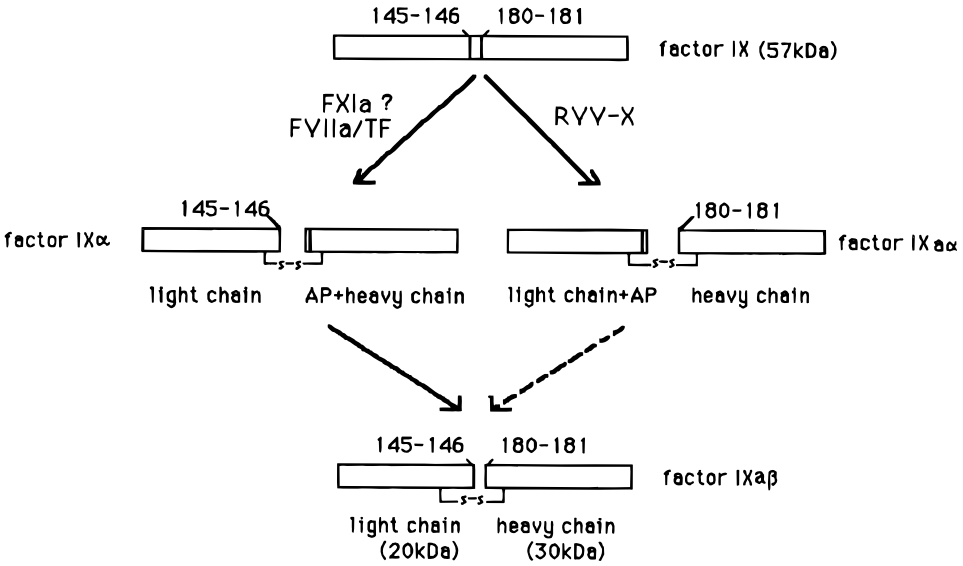

FIGURE 1: Schematic representation of factor IX activation pathways. Factor IX cleavages occur between residues 145 and 146 (resulting in factor IXα), residues 180 and 181 (resulting in factor IXaα), or both (resulting in factor IXaβ). The factor VIIa/tissue factor complex-catalyzed activation proceeds via the factor IXα intermediate. Russell's Viper Venom-X produces primarily factor IXaα; however, the protease also generates a significant amount of factor IXaβ during the reaction. We are unable to determine from our experiments which cleavage occurs first in factor XIa-catalyzed activation.

that the factor IX activation rate increases slightly in the presence of phospholipids (Mannhalter *et al*., 1984). Factor XIa requires calcium for factor IX activation, although magnesium ions increase the factor IX activation rate when either physiological or higher concentrations of calcium are used (Sekiya *et al*., 1995).

In contrast to factor IX activation by the factor VIIa/tissue factor complex, the mechanism of factor IX activation by factor XIa is disputed. Using human factor IX, Osterud *et al*. (1978) observed no intermediate development during the factor XIa-catalyzed activation process. They concluded that the first cleavage made in factor IX is the rate-limiting step in the activation event and that the second cleavage occurs rapidly, with no apparent intermediate formation. Conversely, using both human and bovine factor IX, other groups observed the factor IXα intermediate during the activation reaction and concluded that factor IX activation requires production of a singly-cleaved free factor IXα intermediate (DiScipio *et al*., 1978; Fujikawa *et al*., 1974; Lindquist *et al*., 1978). None of these studies, however, address the possibility that factor IX activation by factor XIa proceeds via a processive mechanism.

We present a study of the activation of plasma-purified human factor IX by factor XIa in which no intermediate development during the reaction course was observed at a low substrate to enzyme ratio (44:1 mol/mol), and in which intermediate development observed at a high substrate to enzyme ratio (1900:1 mol/mol) was inconsistent with a mechanism involving an obligate free intermediate. Our results suggest that factor XIa activates factor IX via a different mechanism of activation than is observed by factor VIIa/tissue factor. Whereas factor VIIa/tissue factor activates factor IX in a nonprocessive mechanism via the long-lived free factor IXα intermediate, factor XIa appears to activate factor IX using a processive mechanism in which both proteolytic cleavages are made rapidly during a single association between the enzyme and substrate so that no free intermediate is generated. The differences in activation mechanisms may explain the presence of multiple physiological activators of factor IX and may help to distinguish the relative mechanisms of action of the extrinsic and intrinsic pathways of blood clotting.

MATERIALS AND METHODS

Materials. Human factor XIa, human factor VIIa, human factor Xa, and human factor IXaα were purchased from Enzyme Research Laboratories (South Bend, IN). Human plasma factor IX was purchased from Enzyme Research Laboratories or was a generous gift from Dr. Kenneth J. Smith (Smith & Ono, 1984). Recombinant tissue factor was a gift from Genentech Inc. (San Francisco, CA). Phosphatidylcholine and phosphatidylserine were purchased from Avanti Polar Lipids, Inc.

Three monoclonal antibodies were used in this study: A-7, A-1, and A-2. Antibody A-7 is divalent metal ion-dependent and recognizes residues 33−40 of factor IX (Cheung *et al*., 1995). Antibodies A-1 and A-2 recognize human factor IX at residues 147−153 (activation peptide) and 180−310 (heavy chain), respectively (Frazier *et al*., 1989).

Factor IX Activation by Factor XIa. Factor IX and factor XIa (at concentrations indicated in the figure legends) were incubated in TBS and 5 mM $CaCl_2$ at 37 °C. Aliquots were drawn, quenched in SDS−PAGE loading buffer with β-mercaptoethanol, and analyzed immediately or stored at −20 °C until analysis.

Factor IX Activation by Factor VII/Tissue Factor. Lipid vesicles containing 70% phosphatidylcholine (PC) and 30% phosphatidylserine (PS) were made using a variation of the method of Hope *et al*. (1985). Briefly, lipids were combined, dried under nitrogen, lyophilized, and resuspended in TBS containing 0.1 mM EDTA. The lipids were then extruded through a 0.2 μm filter 10 times and stored at 4 °C under nitrogen for no more than 2 weeks before using. Factor VIIa (10 nM) and tissue factor (3.3 nM) were preincubated with PS/PC vesicles (10.4 μM) in TBS plus 5 mM $CaCl_2$ at room

temperature for 30 min. Factor IX (1.8 μM) was added, and aliquots were removed, quenched in SDS−PAGE loading buffer with β-mercaptoethanol, and analyzed immediately or stored at −20 °C until analysis.

*Generation of Factor IX*α. Factor IX (1.7 μM) and factor Xa (400 nM) were incubated in TBS with 5 mM $CaCl_2$ at 37 °C for 12 h. Conversion of factor IX to factor IXα was judged to be greater than 90%, based on gel analysis. Residual factor Xa in the factor IXα preparation had no affect in subsequent reactions (data not shown).

*Competition between Factor IX Zymogen and Factor IX*α. Factor IX zymogen (500 nM) and factor IXα (500 nM) were incubated with factor XIa (4.7 nM) in TBS and 5 mM $CaCl_2$ at 37 °C. Aliquots were removed and quenched in SDS−PAGE loading buffer containing β-mercaptoethanol.

*Comparison of Factor IX Zymogen, Factor IX*α*, and Factor IXa*α *Conversion Rates to Factor IXa*β. Factor IX zymogen, factor IXα or factor IXaα (500 nm), and factor XIa (4.7 nM) were incubated at 37 °C in TBS and 5 mM $CaCl_2$. Aliquots were removed and quenched in SDS−PAGE loading buffer (with β-mercaptoethanol for factor IXα).

Iodination of Protein. One hundred micrograms of monoclonal antibody was labeled with 1 mCi of $Na^{125}I$ (Amersham) using Iodobeads according to the manufacturer's directions (Pierce). Labeled antibody was purified from free $Na^{125}I$ using a Sephadex G-25 column.

Detection of Factor IX and Its Cleavage Products. Samples were boiled prior to being loaded onto SDS−PAGE (Laemmli, 1970). The proteins were then either silver-stained or transferred to Immobilon-P transfer membrane (Millipore) for 2 h at 4 °C and 60 V in transfer buffer (25 mM Tris, pH 8.6, 192 mM glycine, and 15% methanol). To ensure that transfer was complete, Immobilon membranes were stained with Coomassie Brilliant Blue and destained prior to blocking. The membranes were blocked with 5% Carnation dry milk in TBS-T, probed with the radiolabeled antibodies in TBS-T containing 5 mM $CaCl_2$ (for A-7 probes) or 10 mM EDTA (for A-1 and A-2 probes), washed in TBS-T (containing $CaCl_2$ or EDTA), and exposed on a Phosphor Screen (Molecular Dynamics). Each membrane was probed successively with antibodies A-7, A-2, and A-1 for identification of all factor IX species. The membranes were stripped between subsequent antibody probes using 62.5 mM Tris, pH 6.7, 2% SDS, and 100 mM β-mercaptoethanol at 50 °C for 30 min.

Data Analysis. The intensity of each band detected by the phosphorimager (Molecular Dynamics) or densitometer (Molecular Dynamics) was determined using the program ImageQuant. The total intensity of each lane was summed, and the percent protein present in each band was calculated and analyzed.

RESULTS

Activation of Factor IX. Neither antibody A-1 (data not shown) nor antibody A-2 (Figure 2a,b) detected a band corresponding to the factor IXα intermediate at low substrate to enzyme (44:1 mol/mol) conditions, using factor IX and factor XIa concentrations below the reported K_m of 0.37 μM (in 5 mM $CaCl_2$) (Sinha *et al*., 1987). In contrast, factor IX activation by factor VIIa/tissue factor was similar to that observed previously (Lawson & Mann, 1991) (Figure 3a,b),

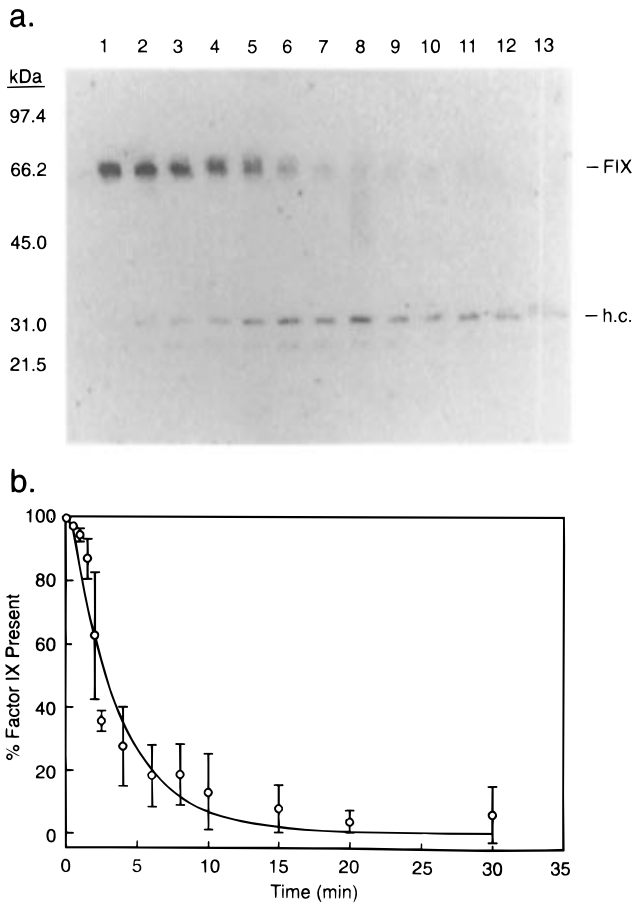

FIGURE 2: Factor XIa activation of factor IX at a low substrate to enzyme ratio (44:1 mol/mol). Factor IX (0.14 μM) and factor XIa (3.1 nM) were incubated in TBS and 5 mM $CaCl_2$ at 37 °C. (a) Phosphorimage of a Western blot membrane probed with the anti-heavy-chain antibody A-2. Lanes 1−13 depict aliquots drawn and quenched at 0, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10, 15, 20, and 30 min, respectively. Because the membrane was first probed with A-7 antibody and then stripped and reprobed with A-2 antibody, some remaining A-7 antibody bound to the factor IX light chain appears as a faint band below the heavy chain. The factor IX zymogen (FIX) and heavy chain (h.c.) are labeled. (b) Graph of the relative concentration of the zymogen (○) present during the reaction course. Quantitation was performed as described under Materials and Methods. Results shown represent the means (±ranges) of four independent experiments. The data were fit to the equation for a first-order exponential function.

in which substantial factor IXα intermediate was detected prior to the appearance of the product.

*Competition between Factor IX Zymogen and Factor IX*α. If the reaction proceeds through an efficiency-enhanced distributive mechanism in which the singly-cleaved intermediate species is a better substrate than factor IX zymogen, the intermediate will be converted to product rapidly and therefore not be detected. To examine this possibility, factor IX zymogen and factor IXα were combined and incubated with factor XIa. The factor IXα concentration decreased at approximately the same rate as factor IX zymogen (data not shown). Thus, the conversion of factor IXα to factor IXaβ was equal to that of factor IX zymogen to factor IXaβ, which is incompatible with an efficiency-enhanced distributive mechanism.

*Comparison of the Factor IX Zymogen, Factor IX*α*, and Factor IXa*α *Conversion Rates*. To further evaluate the possibility of an efficiency-enhanced distributive mechanism,

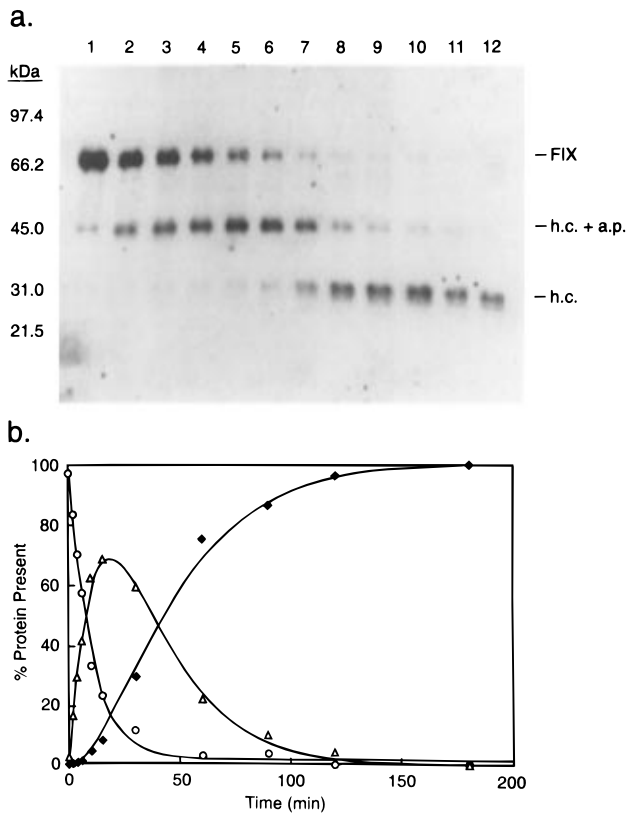

FIGURE 3: Factor VIIa/tissue factor activation of factor IX. The experiment and quantitation of results were performed as described under Materials and Methods. (a) Phosphorimage of a Western blot membrane probed with antibody A-2. Lanes 1−12 depict aliquots drawn at 0, 2, 4, 6, 10, 15, 30, 60, 90, 120, 180, and 240 min, respectively. The factor IX zymogen (FIX), heavy chain with attached activation peptide (h.c. + a.p.), and heavy chain alone (h.c.) are labeled. (b) Graph of the relative concentrations of factor IX zymogen (○), factor IXα intermediate (△), and factor IXaβ (♦) present during the factor VIIa/tissue factor-catalyzed activation reaction. The data were fit to equations described by Lawson and Mann (1991).

the factor XIa cleavage rates of factor IX zymogen (Figures 4a,b), factor IXα (Figure 4a), and factor IXaα (Figure 4b) were compared under identical conditions in which essentially no factor IXα intermediate was observed during zymogen activation (Figure 4a, lanes 2−8). All three activation rates were estimated using the integrated Henri−Michaelis−Menten equation (Segel, 1993). In contrast to the requirements of the efficiency-enhanced distributive mechanism, both the factor IXα and factor IXaα concentrations decreased at rates similar to factor IX zymogen (Figure 4c). The conversion rate constants for factor IX zymogen, factor IXα, and factor IXaα to the product, factor IXaβ, at these conditions were 0.38 ± 0.02 s^{-1}, 0.34 ± 0.05 s^{-1}, and 0.27 ± 0.01 s^{-1}, respectively.

Intermediate Development during the Activation Reaction. At high substrate to enzyme ratios, we sometimes observed the appearance of the factor IXα intermediate. In reactions using a 1900:1 (mol/mol) factor IX to factor XIa ratio, with a factor IX concentration 9 times the reported K_m, some factor IXα development was observed during the reaction course (Figure 5a). However, trace amounts of factor IXα were present in the reaction starting sample, and factor IXaβ developed concomitant with additional factor IXα development (Figure 5b). This is contrary to what was observed in the nonprocessive activation by factor VIIa/tissue factor

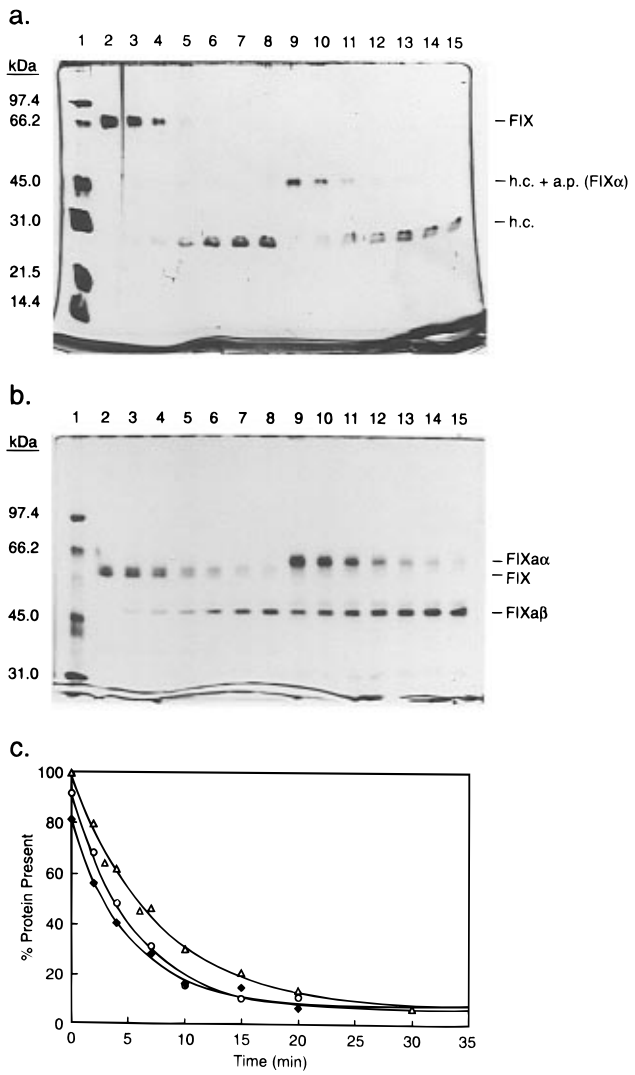

FIGURE 4: Conversions of factor IX zymogen, factor IXα, and factor IXaα to factor IXaβ. Factor IX zymogen, factor IXα or factor IXaα (500 nM), and factor XIa (4.7 nM) were incubated at 37 °C in TBS and 5 mM $CaCl_2$. (a) Silver-stained gel comparing factor IX zymogen and factor IXα conversions to factor IXaβ. Lane 1 shows molecular weight markers. Lanes 2−8 and 9−15 show factor IX zymogen and factor IXα conversions, respectively. Aliquots from each reaction were drawn and quenched in SDS−PAGE loading buffer containing β-mercaptoethanol at 0, 2, 4, 7, 10, 15, and 20 min. (b) Silver-stained gel comparing factor IX zymogen and factor IXaα conversions to factor IXaβ. Lane 1 shows molecular weight markers. Lanes 2−8 and 9−15 show factor IX zymogen and factor IXaα conversions, respectively. Factor IXaα migrated slightly slower than factor IX zymogen, like due to conformational changes after cleavage between residues 180 and 181. Some factor IXaβ (20% of total) was present in the starting sample of factor IXaα. Aliquots from each reaction were drawn and quenched in nonreducing SDS−PAGE loading buffer at 0, 2, 4, 7, 10, 15, and 20 min. (c) Graph showing decreasing factor IX (○), factor IXα (△), and factor IXaα (♦) during the reaction course. Results shown represent the means of two independent experiments. Quantitation was performed as described under Materials and Methods.

(compare Figures 3b and 5b) and to what would be expected from an efficiency-enhanced distributive mechanism.

DISCUSSION

The role of the intrinsic coagulation pathway has been questioned because individuals lacking the intrinsic pathway initiators (factor XII, prekallikrein, high molecular weight

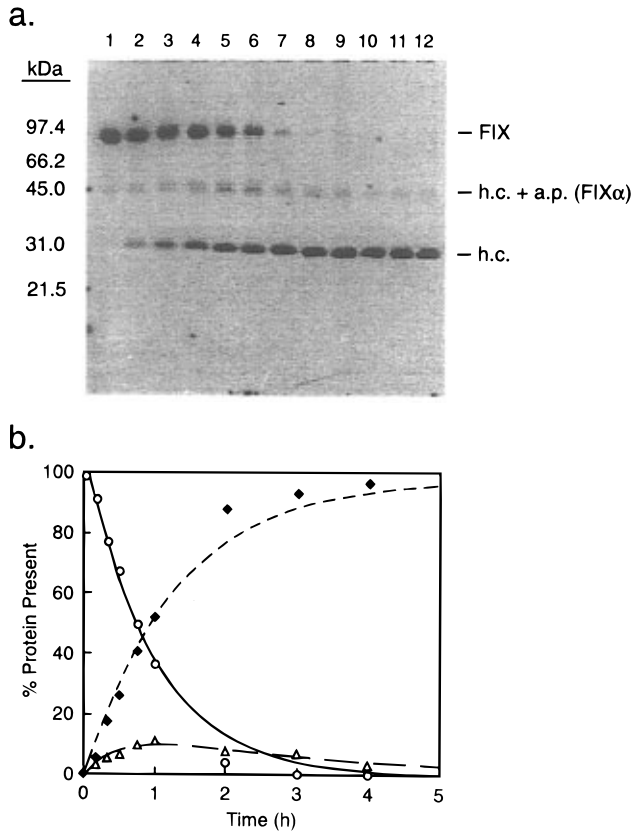

FIGURE 5: Factor XIa activation of factor IX at a high substrate to enzyme ratio (1900:1 mol/mol). Factor IX (1.8 μM) and factor XIa (0.94 nM) were incubated with TBS and 5 mM $CaCl_2$ at 37 °C. (a) Phosphorimage of a Western blot membrane probed with antibody A-2. Lanes 1−12 depict aliquots drawn and quenched at 0, 10, 20, 30, 45, and 60 min, and at 2, 4, 6, 8, 10, and 24 h, respectively. (b) Graph showing the changing concentrations of factor IX zymogen (○), factor IXα (△), and factor IXaβ (♦) between 0 and 5 h of the reaction course. The data were fit to the equations described by Lawson and Mann (1991).

kininogen, and factor XI) do not typically exhibit a bleeding diathesis. Indeed, the need for multiple physiological activators of factor IX is perplexing, particularly when factor VIIa/tissue factor can effectively generate factor IXaβ and lead to thrombin development (Jones & Mann, 1994; Lawson & Mann, 1991). However, our study of factor IX activation by factor XIa indicates that intrinsic activation proceeds differently than extrinsic activation and therefore may function in a different capacity than factor VIIa/tissue factor-catalyzed activation.

In our hands, factor IX activation by factor XIa proceeded without the development of the factor IXα intermediate when using a low substrate to enzyme ratio (44:1 mol/mol). Given the sensitivity of our detection system, only two mechanisms could explain the absence of an intermediate in the activation reaction. First, it is possible that factor XIa makes one cleavage and releases the singly-cleaved intermediate which then rapidly reassociates with the enzyme and is converted to product, so that the intermediate is not detected (an efficiency-enhanced distributive mechanism) (Morris *et al.*, 1995). Since no intermediate was observed in the reactions, the free intermediate to product conversion rate would have to be *at least 100-fold faster than* the zymogen to intermediate conversion rate to comply with the efficiency-enhanced distributive mechanism. Alternatively, the reaction may proceed via a processive mechanism, in which there is a

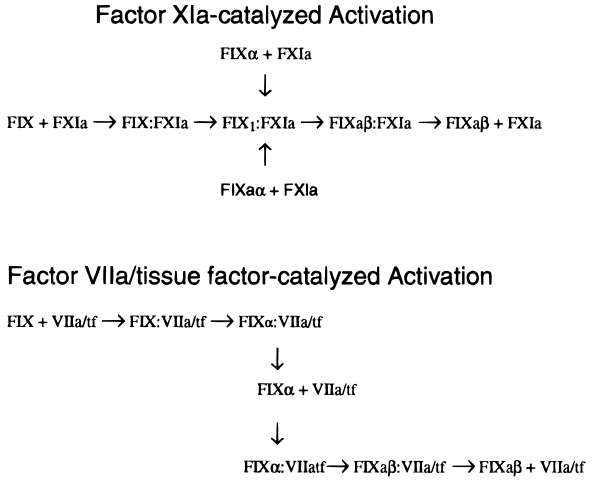

FIGURE 6: Comparison between the factor XIa- and factor VIIa/tissue factor-dependent mechanisms of factor IX activation. Proteins are abbreviated as follows: FIX (factor IX), FXIa (factor XIa), FIXα (factor IXα), FIXaα (factor IXaα), FIX_1 (singly-cleaved factor IX), FIXaβ (factor IXaβ), VIIa/tf (factor VIIa/tissue factor). In contrast to factor VIIa/tissue factor-catalyzed activation, factor XIa-catalyzed activation of factor IX proceeds without dissociation between the enzyme and substrate complex during the reaction course.

single association between the substrate and the enzyme during which both cleavages are made. To test the efficiency-enhanced distributive mechanism, we combined equimolar amounts of free factor IXα and factor IX zymogen with factor XIa and observed that factor IXα was cleaved at a rate similar to that of factor IX zymogen. Additionally, we studied the factor XIa cleavages of free factor IXα and factor IXaα. The cleavage rates of both free intermediates were essentially identical to that of factor IX zymogen. According to the calculated rates for free factor IXα and factor IXaα conversion, approximately 42% intermediate should have accumulated during the reaction course; however, we did not observe significant intermediate accumulation. These results strongly suggest that factor XIa-catalyzed activation does not proceed via a free factor IXα or factor IXaα species.

When we used a high factor IX to factor XIa ratio (1900:1 mol/mol), a small but significant amount of factor IXα development was observed. In the case of a nonprocessive reaction, such as that observed during extrinsic activation, factor VIIa first makes one cleavage in factor IX and releases the factor IXα intermediate. Thus, the intermediate appears prior to the product (Figure 3a,b). Even when an efficiency-enhanced distributive mechanism is operating, the obligate intermediate must appear prior to the product. However, during factor XIa cleavage of factor IX, the intermediate (factor IXα) developed *with* and not *prior to* generation of the factor IXaβ product (Figure 5a,b). Indeed, the codevelopment of factor IXα and factor IXaβ was also observed in several earlier papers (DiScipio *et al.*, 1978; Monroe *et al.*, 1989). These observations are inconsistent with an efficiency-enhanced distributive model. On the other hand, if a processive enzyme undergoes a consistent, periodic failure and makes only one cleavage before releasing a singly-cleaved intermediate, we would observe the intermediate concentration to initially rise with, and not prior to, the product. Thus, the observed behavior at a high substrate to enzyme ratio is consistent with a processive mechanism displaying a low failure rate.

The mechanism shown in Figure 6 describes the factor XIa-dependent activation of factor IX. In contrast to the factor VIIa/tissue factor-catalyzed reaction which proceeds via the free factor IXα intermediate, there is no dissociation between the substrate and enzyme during the factor XIa-catalyzed reaction. Furthermore, because of the similarity between factor XIa-catalyzed conversion rates of factor IX zymogen and free factor IXα and factor IXaα, the rate-limiting step is probably common to all three reactions. Interestingly, we observed a small but reproducible lag in the data for factor XIa-catalyzed activation (Figure 2b). However, we were unable to determine whether this lag represents part of the activation mechanism.

Gailani and Broze (1991) and Davie *et al*. (1991) each proposed a revised version of the clotting cascade in which the factor VIIa/tissue factor complex initiates coagulation by generating small amounts of factor Xa and factor IXa but is subsequently rapidly inhibited by tissue factor pathway inhibitor (TFPI). Factor Xa and factor IXa then generate thrombin which activates cofactors V and VIII and maintains sufficient coagulation activity to cause fibrin polymerization and cessation of bleeding. However, under more traumatic conditions or in tissues with high fibrinolytic activity, the intrinsic pathway must be initiated to supplement the production of factor IX and provide a sustained response necessary for hemostasis. Thus, a different mechanism of factor IX activation could provide an alternate means for regulation or control of the clotting events. Lawson and Mann (1991) demonstrated that factor VIIa/tissue factor converts the factor Xa-generated factor IX intermediate, factor IXα, to factor IXaβ more rapidly than it does factor IX zymogen. Furthermore, Mast and Broze (1996) recently showed that free factor Xa is rapidly inhibited by TFPI. Thus, the effective concentrations of free factor Xa around the injury site may be rapidly reduced after a trauma, limiting the amount of factor Xa available for factor IXα generation, and emphasizing the need for an alternate factor IX-activating enzyme. An enzyme such as factor XIa which can efficiently make both necessary cleavages in factor IX (without requiring factor Xa-catalyzed preconversion to factor IXα) might therefore satisfy this need. Since factor XIa has two identical active sites, it may be able to cleave factor IX at two spatially distinct sites without requiring a conformational change between successive cleavages. This alternate mechanism of factor IX activation may therefore provide an opportunity for a different method of regulation specific to the factor XIa/factor IX complex, which is essential for the necessary control of coagulation.

In conclusion, our results indicate that factor XIa-catalyzed activation of factor IX proceeds via a processive mechanism without free factor IXα intermediate generation. This mechanism differs from that previously suggested and from factor VIIa/tissue factor-catalyzed activation. We suggest that this processive mechanism provides a means by which factor IX may be efficiently activated in the event of intrinsic pathway activation after a severe trauma.

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