Factors IXa and Xa Play Distinct Roles in Tissue Factor-Dependent Initiation of Coagulation

By Maureane Hoffman, Dougald M. Monroe, Julie A. Oliver, and Harold R. Roberts

Tissue factor is the major initiator of coagulation. Both factor IX and factor X are activated by the complex of factor VIIa and tissue factor (VIIa/TF). The goal of this study was to determine the specific roles of factors IXa and Xa in initiating coagulation. We used a model system of in vitro coagulation initiated by VIIa/TF and that included unactivated platelets and plasma concentrations of factors II, V, VIII, IX, and X, tissue factor pathway inhibitor, and antithrombin III. In some cases, factor IX and/or factor X were activated by tissue factor-bearing monocytes, but in some experiments, picomolar concentrations of preactivated factor IX or factor X were used to initiate the reactions. Timed samples were assayed for both platelet activation and thrombin activity. Factor Xa was 10 times more potent than factor IXa in initiating platelet activation, but factor IXa was much more effective in promoting thrombin generation than was factor Xa. In the presence of VIIa/TF, factor X was required for both platelet activation and thrombin generation, while factor IX was only required for thrombin generation. We conclude that VIIa/TF-activated factors IXa and Xa have distinct physiologic roles. The main role of factor Xa that is initially activated by VIIa/TF is to activate platelets by generating an initial, small amount of thrombin in the vicinity of platelets. Factor IXa, on the other hand, enhances thrombin generation by providing factor Xa on the platelet surface, leading to prothrombinase formation. Only tiny amounts of factors IX and X need to be activated by VIIa/TF to perform these distinct functions. Our experiments show that initiation of coagulation is highly dependent on activation of small amounts of factors IXa and Xa in proximity to platelet surfaces and that these factors play distinct roles in subsequent events, leading to an explosion of thrombin generation. Furthermore, the specific roles of factors IXa and Xa generated by VIIa/TF are not necessarily reflected by the kinetics of factor IXa and Xa generation.

© 1995 by The American Society of Hematology.

EXPOSURE OF blood to tissue factor is the major physiologic stimulus to initiate coagulation. Coagulation factor VIIa associates with tissue factor, and the complex activates both factor X and factor IX. Activated factor X (Xa) reacts with and is inhibited by tissue factor pathway inhibitor (TFPI). The TFPI/Xa complex then inhibits the VIIa/TF complex. Thus, in order for coagulation to occur, sufficient amounts of procoagulant factors must be generated during a limited period of time when the VIIa/TF complex is active. The regulation of tissue factor exposure and activity is crucial to controlling initiation of coagulation. Even though platelets provide the major procoagulant surface for hemostasis, they do not express tissue factor and, therefore, cannot initiate the process. Activated coagulation factors produced at the site of VIIa/TF activity must find their way to the platelet surface to effectively propagate coagulation. Thus, induction of coagulation by tissue factor is a complex process. Its success or failure is governed by spatial, temporal, and quantitative features of factor IXa and factor Xa generation (ie, where, when, and how much factor is activated).
factor IX or factor X was omitted. In the second type of experiment, we added purified factor IXa or factor Xa to activated or unactivated platelets suspended in a mixture of purified zymogen coagulation factors, TFPI, and AT. In the latter experimental design, coagulation was initiated by the addition of specific amounts of preactivated factors IX and/or X, rather than allowing tissue factor to activate them. This experimental design allowed us to isolate the effects of factors IXa and Xa on platelet activation and thrombin generation.

In our system, platelet activation always preceded thrombin generation. The addition of preactivated factor X efficiently promoted platelet activation but led to a relatively small amount of subsequent thrombin generation. Preactivated factor IX was an order of magnitude less potent than factor Xa in inducing platelet activation, but once platelet activation had occurred, factor IXa was much more effective than factor Xa at promoting thrombin generation. Omission of factor X from a VIIa/TF-initiated system (containing tissue factor-bearing cells, small amounts of factor VIIa, unactivated platelets, and plasma levels of zymogen factors II, V, VIII, IX, and X) eliminated both platelet activation and thrombin generation. Omission of factor IX from this system (mimicking hemophilia B) dramatically reduced thrombin generation, while platelet activation occurred at a nearly normal rate. These experiments support the conclusion that factor Xa and factor IXa activated by VIIa/TF play different and distinct roles in initiating coagulation. The initial activation of factor X, which occurs before platelet activation, is more important for generating the first small amounts of thrombin required for platelet activation. Factor IX activation plays a major role in determining the rate of thrombin formation by activating factor X on the platelet surface, where it can assemble with factor Va into active prothrombinase complexes that lead to the ultimate explosion of thrombin generation.

MATERIALS AND METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (Grand Island, NY). Chromozyme TH was purchased from Boehringer-Mannheim (Indianapolis, IN). Spectrozyme FXa and TenStop were purchased from American Diagnostica (Greenwich, CT). Recombinant hirudin was purchased from Accurate Chemical and Scientific Corp (Westbury, NY). Antihuman factor X and antihuman factor IX polyclonal rabbit antibodies were purchased from Dako Corporation (Carpenteria, CA). Anti-CD62 (phycocerythrin-conjugated) was purchased from Becton Dickinson (San Jose, CA). Mono-Poly Resolving Medium was purchased from Flow Laboratories, Inc. Lipopolysaccharide (LPS) from Escherichia coli was purchased from Sigma Chemical Co (St Louis, MO). All other chemicals were of high commercial grade.

Proteins. Prothrombin and factor IX were purified from human plasma as described previously.11 Factor X was purchased from Enzyme Research Laboratories (South Bend, IN), and factor V from Calbiochem Corporation (La Jolla, CA) or HaematoLogic Technologies (Essex Junction, VT). Factor VIII was purchased from the University of North Carolina hospital pharmacy as Profilate (Alpha Therapeutics, Los Angeles, CA). Thrombin12 and AT13 were purified from human plasma as previously described. Prothrombin, factor X, and factor IX were treated with an inhibitor mixture (1 μmol/L tosyl-Lysyl-chloromethyl ketone, tosyl-Phenyl-chloromethyl ketone, Gly-Arg-chloromethyl ketone, Phe-Pro-Arg-chloromethyl ketone, and Phenyl-methyl-sulfonyl fluoride), followed by exhaustive dialysis to inactivate proteases in the zymogens. In addition to treatment with low-molecular-weight inhibitors, the zymogen proteases were incubated for 12 hours with AT at a concentration 10 times that in plasma. The proteins were diluted to plasma concentration just before the experiment. Recombinant factor VIIa and recombinant TFPI were gifts of NOVO Nordisk (Gentofte, Denmark). Factors IXa and Xa were prepared from the respective zymogens as previously described.14 Briefly, factor IXa was activated with factor Xla (Enzyme Research Laboratories), and factor X with purified coagulant protein from Russell’s viper venom (HaematoLogic Technologies). The activated factors were re purified on Q-Sepharose with calcium chloride elution.15

Cell isolation. Monocytes were purified on Ficoll-Hypaque density gradients from the blood of healthy volunteers.16 The mononuclear cell band was collected and diluted in an equal volume of 13 mmol/L citrate (pH 7.4), 123 mmol/L NaCl, 33 mmol/L dextrose  with 10 mmol/L prostaglandin E1. The nucleated cells were sedimented at 100g for 5 minutes, and the platelet-containing supernatant was retained for gel filtration.17 The mononuclear cell-containing pellet was washed twice with cold calcium-free medium, and the resulting pellet was resuspended in DMEM with 1 mg/mL dextrose and no serum. Two thousand monocytes per well were plated in 96-well plates in DMEM containing 0.5 μg/mL bacterial LPS. The plates were incubated at 37°C in 5% CO2 for 1 hour. Nonadherent cells were removed by washing three times with fresh DMEM. Fresh LPS-containing DMEM was added, and the cells were cultured for 18 hours. The LPS-treated monocyte monolayers (LPS-monocytes) were then washed three times with Tyrodes buffer (15 mmol/L Hepes, pH 7.4, 33 mmol/L NaPO4, 138 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L MgCl2, 5.5 mmol/L dextrose) containing 1 mg/mL bovine serum albumin (Tyrodes/albumin) before being used in experiments. The adherent population after culture and washing consisted of greater than 95% monocytes, with less than 5% contamination with lymphocytes. Platelet contamination was from one to two platelets per monocyte. This level of contamination was quite low compared with the number of purified platelets added to reaction wells (<0.006% of the platelets added).

Platelets were separated from plasma proteins by gel-filtering the platelet-containing supernatant from the monocyte preparation over a 50-mL column of Sepharose CL-2B in Tyrodes/albumin. The platelet preparations contained less than 0.1% contamination by leukocytes. Platelets were kept at 37°C until used. Platelet activation, defined as expression of the CD62 antigen (P-selectin, GMP-140), was measured by flow cytometry.18 Reproducibly, fewer than 10% of the platelets were activated by the isolation procedure.

Experimental assay system. The complete cell and protein system is a modification of one we have previously used to examine tissue factor-initiated procoagulant complex assembly on the platelet surface.19 Tissue factor-bearing monocytes were incubated with purified coagulation proteins or proteins and platelets for up to 16 minutes. The platelets, isolated from the same blood donor as the monocytes, were at a final concentration of 80,000/μL to 110,000/μL. The final concentrations of proteins were selected to approximate plasma levels: factor X, 8 μg/mL; factor IX, 4 μg/mL; factor VIII, 1 μg/mL; factor V, 7 μg/mL; TFPI, 0.1 μg/mL; and AT, 150 μg/mL. The concentration of prothrombin used, 50 μg/mL, is at the lower limit of normal plasma concentrations for this factor. The reactions were initiated by the addition of factor VIIa (0.01 μg/mL) and calcium (3 mmol/L). In some experiments, either factor IX or factor X was omitted from the protein mixture incubated with monocytes and platelets. In these experiments, 100 μg/mL of rabbit polyclonal anti-factor IX or anti-factor X (both from Dako Corp, Santa Barbara, CA) was included in the incubation mixture to inactivate any trace
of the omitted factor. Although the system is artificial, it was designed to mimic the initial in vivo coagulation reactions.

In other experiments, different amounts of activated factor IX and/or factor X were added directly to a mixture of platelets, coagulation proteins, and inhibitors except factor VIIa. Once the proteins had been added to the platelets, samples were taken at timed intervals and assayed for thrombin activity and platelet activation. The amount of thrombin generated in any given set of experiments was a function of the individual whose cells were used in the experiments. In replicate experiments, the pattern of platelet activation and the relative amounts of thrombin generated with different treatments were consistent, even though the absolute amounts of thrombin generated were different for cells from different donors. Each figure shows data run in parallel from one individual and is representative of experiments using cells from other individuals.

**Assays.** For the assay of thrombin activity, 10-μL samples from the experimental wells were added to 90 μL of 0.45 mmol/L Chromozyme TH, 30 μmol/L/TrisStop (sufficient to block the greatest amount of factor Xa produced in these experiments), 2.2 mmol/L EDTA, in 20 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 1 mg/mL albumin. The assay mixture was incubated for 13 minutes, then cleavage of the chromogenic substrate was terminated by addition of 100 μL of 20% acetic acid. The amount of active enzyme was related to the cleavage of the synthetic substrate (measured at 405 nm in a Vmax plate reader [Molecular Devices Corp., Menlo Park, CA]) by a standard curve made using purified thrombin.

For assays of platelet activation and factor Xa binding, separate 8-μL samples from the reaction mixtures were removed and added to 30 μL of 2% paraformaldehyde in 20 mmol/L HEPES, 150 mmol/L NaCl, 3 mmol/L CaCl2. Platelets were fixed in paraformaldehyde for 30 minutes, then diluted to 5 mL of Tyrodes/albumin, and incubated for at least an additional 30 minutes. Platelet activation was assessed by measuring expression of the activation-specific alpha granule marker CD62. Samples were stained with phycoerythrin-conjugated anti-CD62 (final dilution, 1:50) for 30 minutes at 25°C. Relative factor Xa binding was assessed as previously described. Briefly, the platelet samples were incubated for 30 minutes with a 1:200 dilution of antihuman factor X (Dako), washed once, then incubated for 30 minutes in a 1:2 dilution of fluorescein isothiocyanate (FITC)-conjugated goat antirabbit IgG (Sigma). The samples were washed once with filtered phosphate-buffered saline (PBS) and resuspended in 0.5 mL PBS, and the mean fluorescence intensity was determined on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

The activation state of the platelet-bound factor X was determined by Western blot analysis. Samples were removed from the incubation mixtures, and the platelets were centrifuged through a layer of 10% sucrose with 3 mmol/L CaCl2, to separate free from bound ligand. The platelet pellets were resuspended in 20 μL of 10 mmol/L EDTA to dissociate membrane-bound factors X and Xa. The platelets were sedimented in a microfuge, and the supernatant was added to an equal volume of sodium dodecyl sulfate (SDS)-containing sample buffer and boiled for 1 minute. The samples were separated by polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes on a Phastgel System (Pharmacia, Uppsala, Sweden). Factor X antigen was detected using the same polyclonal antibody to human factor X as that used by others. We have previously shown that platelet activation is dependent on the generation of small amounts of thrombin, because no platelet activation occurs in the absence of thrombin.

Briefly, the platelet pellets were incubated for 30 minutes with a 1:200 dilution of antihuman factor X (Dako), washed once, then incubated for 30 minutes in a 1:3 dilution of fluorescein isothiocyanate (FITC)-conjugated goat antirabbit IgG (Sigma). The samples were washed once with filtered phosphate-buffered saline (PBS) and resuspended in 0.5 mL PBS, and the mean fluorescence intensity was determined on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

**Results**

**Platelet activation and thrombin generation in the tissue factor-initiated system.** Platelets (100,000/μL final concentration) were incubated with tissue factor-bearing monocytes and plasma levels of purified prothrombin, factor X, factor IX, factor VIII, AT, TFPI, and a catalytic amount of factor VIIa. Platelet activation, as measured by exposure of the alpha granule membrane protein CD62, began within about 2 minutes after the addition of calcium (Fig 1A). However, in the absence of monocytes, very little platelet activation occurred. We have previously shown that platelet activation is dependent on the generation of small amounts of thrombin, because no platelet activation occurs in the absence of thrombin.

The time course of thrombin generation in the presence of platelets and monocytes was compared with thrombin generation in wells containing platelets alone or monocytes alone. As shown in Fig 1B, very little thrombin generation occurred in wells containing the coagulation proteins and platelets only. However, in the presence of both monocytes and platelets, thrombin generation began after a 2- or 3-minute lag period followed by a rapid burst of thrombin generation. Monocytes alone did not support generation of significant amounts of thrombin (data not shown). No thrombin generation was observed in the absence of calcium or when monocytes and platelets were both omitted. In addition, when monocytes were preincubated with an anti-tissue factor antibody at concentrations that completely inhibited monocyte tissue factor activity, platelet activation and thrombin generation were nearly identical to that seen in the absence of monocytes.

**Effect of factor IXa and factor Xa on platelet activation and thrombin generation.** Once we had ascertained that platelet activation and thrombin generation were dependent on the presence of tissue factor-bearing monocytes in a complete system that included platelets and coagulationzymogens, we conducted experiments to determine whether the addition of low concentrations of purified factor IXa, factor Xa, or combinations of the two factors, could reproduce the
FACTORS IXa AND Xa IN TF-INITIATED COAGULATION

Fig 1. (A) Effect of tissue factor-bearing monocytes on platelet activation. Wells contained prothrombin, TFPI, AT, and factors IX, X, VIII, V, and VIIIa incubated with platelets, 100,000/μL, with (2,000/μL) or without monocytes ( ). Platelet activation was measured at the times indicated as described in Materials and Methods and is shown as the percent of platelets expressing CD62. The figure shows data from one representative experiment of 10 performed. (B) Effect of tissue factor-bearing monocytes on thrombin generation. Thrombin generation was measured as described in Materials and Methods. The data are from the same experiment as in panel A and are representative of 10 experiments performed. ( ), monocytes plus platelets; ( ), platelets only.

Fig 2. Effect of factors IXa and Xa on platelet activation. Platelets (160,000/μL) were incubated in wells containing prothrombin, TFPI, AT, and factors V, IX, X, and VIII, with the addition of different concentrations of factor Xa (upper panel) or factor IXa (lower panel). Samples were removed for fixation at the indicated times. Expression of CD62 was measured by flow cytometry as described in Materials and Methods. The data are shown from one experiment and are representative of four performed. ( ), 0 pmol/L; ( ), 1 pmol/L; ( ), 10 pmol/L; ( ), 100 pmol/L.

Effects of tissue factor on platelet activation and thrombin generation. Different concentrations of factors IXa and Xa were added to wells containing platelets, all the zymogen coagulation factors, inhibitors, and calcium. The effect of factors IXa and Xa on platelet activation is shown in Fig 2. We found that as little as 10 pmol/L factor Xa (0.008% of the plasma concentration of factor X) was sufficient to initiate platelet activation in the presence of the other zymogen coagulation factors (Fig 2, upper panel). However, platelet activation did not occur when prothrombin was omitted from the mixtures. This suggests that small amounts of factor Xa promote platelet activation by activating prothrombin to thrombin in amounts too small to be detected in the supernatant. By contrast, 10 pmol/L factor IXa (0.015% of the plasma IX concentration) had no detectable effect on platelet activation (Fig 2, lower panel). Considerably higher concentrations of factor IXa were required to induce platelet activation. For example, addition of 100 pmol/L factor IXa resulted in 50% of the platelets being activated after about 10 minutes. The addition of 100 pmol/L factor Xa resulted in 50% of the platelets being activated after only 4 minutes. Neither factor IXa nor factor Xa directly activated platelets (data not shown).
A number of different events occur during the process of platelet activation. The platelet activation marker we used, CD62, reflects secretion of alpha granule contents. Alpha granule release is relatively slow compared with other events, such as shape change, activation of glycoprotein IIb/IIIa, and surface exposure of phosphatidyl serine. Thus, while we did not directly measure exposure of procoagulant lipids, CD62 expression is a good indicator that the platelet surface has already undergone the transformation to a procoagulant state. In fact, we found that CD62 expression always preceded large-scale thrombin generation in our model system. Thus, we might expect that exogenous factor Xa would be more effective in promoting thrombin generation than factor IXa, because it is more effective at inducing platelet activation. To separate an effect on platelet activation from an effect specifically on thrombin generation, we measured the amount of thrombin generated when the platelets were activated before the addition of coagulation factors. Platelets were activated by incubation for 10 minutes with 50 μg/mL of a thrombin receptor agonist peptide (SFLLRN), which has no proteolytic activity of its own. This concentration was more than sufficient to induce maximal CD62 expression. Zymogen coagulation factors II and V, inhibitors, and calcium were added to activated platelets. In addition, either factor Xa or factors IXa, VIII, and X were added. The zymogens were always added at concentrations approximating those in plasma as detailed in Materials and Methods, while the activated factors were added at varying, but miniscule, concentrations. As shown in Fig 3, the amount of thrombin generated on activated platelets to which 10 or 100 pmol/L factor IXa had been added (lower panel) was significantly greater than platelets to which 10 or 100 pmol/L factor Xa had been added (upper panel). This finding suggests that factor IXa, in the presence of zymogen coagulation factors, is more effective than the same amount of exogenous factor Xa at promoting thrombin generation. We hypothesized that this is because factor IXa, as part of the Xase complex, can continue to activate factor X and provide a source of platelet-
assessed factor Xa to form prothrombinase complexes. To test this hypothesis, we also measured the amount of platelet-associated factor X(a) resulting from the addition of different amounts of exogenous factor IXa or Xa in the experiments detailed above. As shown in Fig 4, the addition of increasing amounts of factor IXa, in the presence of factors VIII and X, resulted in increasing amounts of factor X antigen associated with the platelet surface, up to twice the amount of factor X bound in the absence of added factor IXa. The polyclonal antibody used in these studies does not distinguish between zymogen and activated factor X. However, the increase in platelet-associated factor X correlated with the rate of thrombin generation, suggesting that addition of factor IXa results in increased functional factor Xa on the platelets. The addition of increasing amounts of factor Xa was not reflected in a significant increase in thrombin generation or platelet-associated factor X, suggesting that much of the exogenous factor Xa was unable to reach the platelet surface, perhaps being bound by inhibitors.

We next examined the activation state of the platelet-bound factor X(a) antigen. Platelets were activated with SFLLRN and then added to a mixture of zymogen coagulation factors II, V, VIII, and X, as well as AT and TFPI. Different amounts of factor IXa were added, and timed samples were removed as in the experiments described above. These samples were immediately centrifuged through sucrose to separate free from platelet-bound factors, and then EDTA was added to the platelet pellets to dissociate bound factor X(a). The eluates were subjected to Western blot analysis. Initially, the great majority of platelet-associated factor X antigen was in the zymogen form. In the presence of added factor IXa, there was a progressive increase in the amount of activated factor X (Fig 5). In the absence of factor IXa, there was no increase in the proportion of platelet-bound factor X in the activated form. Very little of the factor X in the supernatant was activated, and very little antigen was detected at a molecular weight consistent with factor Xa/AT complexes. Thus, both factor X and Xa are bound to activated platelets, but factor IXa promotes the progressive activation of factor X on platelet surfaces. The platelet-associated factor Xa could then be directly incorporated into prothrombinase complexes.

**Factor IX and factor X activation on tissue factor-bearing monocytes.** To measure how much factor X and factor IX was activated on the tissue factor-bearing monocytes, 100,000 monocytes per well were incubated with factor VIIa, calcium, and plasma concentrations of either factor IX or X for 1 hour. The amount of each factor that had been activated was measured by activity assays. We calculated that the amount of the factors activated in 1 minute by the number of monocytes used in our experiments (2,000 per well) would result in no more than 10 pmol/L of each activated factor being transferred to the platelet suspensions.
Roles of factors IXa and Xa in a tissue factor-initiated model system. Finally, we returned to a tissue factor-initiated system to determine whether factor IX and factor X activated on a cell surface functioned similarly to exogenously added factors IXa and Xa. Tissue factor-bearing monocytes and platelets were incubated with the complete complement of coagulation proteins (AT, TFPI, factors II, V, VIIa, VIII, IX, and X) and compared with incubation mixtures lacking either factor IX or factor X. Mixtures lacking factor X demonstrated neither platelet activation nor thrombin generation (Fig 6). Mixtures lacking factor IX showed a nearly normal rate of platelet activation (upper panel) but only a very small amount of thrombin generation (lower panel). Thus, in a tissue factor-initiated system, factor X activation by the extrinsic pathway promotes platelet activation, while factor IX activation by VIIa/TF is required for optimal thrombin generation on the platelet surface.

DISCUSSION

The work described in this report was undertaken to investigate the relative roles of factor IX and factor X activation by tissue factor in the process of initiating coagulation. One of the unanswered questions in hemostasis is why a deficiency of factor IX or factor VIII can cause such a severe bleeding diathesis. These factors work together to form the platelet (intrinsic) Xase complex. However, the VIIa/TF complex should be able, at least partially, to offset a deficiency of factor IX or VIII by activating factor X itself. As factor IX is a competitive inhibitor of factor X activation by VIIa/TF, one might expect tissue factor-dependent activation of factor X to be increased in a compensatory manner when factor IX is lacking. A number of investigators have addressed this problem by studying the kinetic features of factor IX and X activation by tissue factor. Others have suggested that VIIa/TF cannot compensate for a lack of factor IX because TFPI rapidly inactivates it once some factor Xa has been generated. However, kinetic studies of the activities of tissue factor and its inhibitor do not necessarily address questions related to the physiology of the system. Our studies were designed to look at the effects of the products of VIIa/TF activity on platelets and platelet surface coagulation complexes as a step toward determining whether factors IXa and Xa, activated by VIIa/TF, play distinct physiologic roles in the clotting process.

Our data suggest that factor IX and factor X activated by VIIa/TF play distinct roles in coagulation. Exogenously added factor Xa efficiently promoted platelet activation, and, in a tissue factor-initiated system, factor X was required for platelet activation and thrombin generation. However, once platelets were activated, the amount of thrombin generated was not a function of the amount of exogenously added factor Xa. We conclude that factor X activation on a tissue factor-bearing cell leads to the generation of small amounts of thrombin, which then leads to platelet activation.

In contrast to the effects of factor Xa, exogenously added factor IXa had little ability to promote platelet activation. However, added factor IXa led to increasing amounts of factor Xa on the platelet surface, ultimately leading to a burst of thrombin generation. In our tissue factor-initiated model system, omission of factor IX allowed nearly normal platelet activation but only minimal thrombin generation. We conclude that factor IX activation is not required for platelet activation but is required for optimal thrombin generation. Our results suggest that this is because factor IXa, in complex with factor VIIIa, provides an ongoing supply of factor Xa on the platelet surface for formation of the prothrombinase complex. Factor Xa added into the
fluid phase at the beginning of the reaction (as provided by VIIa/TF) is not as effective in generating thrombin as is factor Xa generated on the platelet surface by the factor IXa/VIIa complex. There are probably at least two reasons for this. First, factor Va constitutes part of the platelet surface-binding site for factor Xa. Factor Xa that is formed by VIIa/TF before platelets and factor Va are activated has no means of localizing on the platelet surface. Instead, this factor Xa remains in solution, where it is susceptible to inhibition by AT and TFPI. Second, factor Xa that is activated by the factor IXa/VIIa complex on the activated platelet may remain localized on the platelet surface and move directly into complex with factor Va. This factor Xa formed by the platelet Xase remains protected from inhibitors and can most efficiently participate in formation of prothrombinase complexes. Thus, factor X activated by VIIa/TF on monocytes, endothelial cells, or stroma is a poor substitute for factor X activated by the platelet factor IXa/VIIa complex. This interpretation is consistent with the fact that factor X activated by VIIa/TF does not substitute for a lack of platelet Xase activity in patients with hemophilia.

Kinetic studies have provided detailed information about the rates of factor IX and factor X activation by VIIa/TF. However, kinetic studies may not address the physiologic roles of factor IX and factor X activation in tissue factor-initiated hemostasis. Our current data, based on an in vitro model that reflects in vivo conditions, suggest that factors IXa and Xa generated by the tissue factor pathway play distinct and complementary roles, and that both are required for effective initiation of coagulation. Optimal thrombin generation is achieved only when both factor X and factor IX are activated by the VIIa/TF complex. Factor Xa promotes platelet activation, probably by activating small amounts of prothrombin. Factor IXa promotes rapid thrombin generation on the activated platelet surface by activating factor Xa, which can then be incorporated into the platelet surface prothrombinase complex.

ACKNOWLEDGMENT

We acknowledge the excellent technical assistance of Suzie Worthing and Missy Martin.

REFERENCES

Factors IXa and Xa play distinct roles in tissue factor-dependent initiation of coagulation

M Hoffman, DM Monroe, JA Oliver and HR Roberts