Activation of Human Factor VII by Factors IXa and Xa on Human Bladder Carcinoma Cells

By Peter Wildgoose and Walter Kisiel

Single chain factor VII is converted by limited proteolysis to its activated form, factor VIIa, by a number of blood coagulation proteases including factor IXa and factor Xa. We have determined the relative rate of human factor VII activation by human factors IXa and Xa in two different systems: one containing Ca\(^{2+}\) and human bladder carcinoma (J82) cells, and the other containing Ca\(^{2+}\) and mixed brain phospholipids. The rate of factor VII activation was determined by a one stage coagulation assay, and proteolytic cleavage of factor VII was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting techniques. On a molar basis, factor Xa was sixfold more efficient than factor IXa in activating factor VII when the activation reaction occurs on J82 cell surfaces. In contrast, when incubation takes place in a suspension of mixed phospholipids, factor Xa was 18-fold more efficient in activating factor VII than factor IXa. In addition, factor IXa activated factor VII at a rate approximately one-half that observed using factor IXa. In the absence of cells or phospholipids, no activation of factor VII by either factors IXa or Xa was observed. The addition of stoichiometric amounts of either recombinant human factor VIII (des B-domain) or plasma-derived factor VIIa failed to augment the rate of factor VII activation by either factors IXa or IXa. Likewise, purified human factor Xa failed to influence the rate of factor VII activation by factor Xa in either system. Collectively, our studies reveal that J82 cells possess procoagulant phospholipid capable of readily supporting the activation of factor VII by either factors IXa or Xa. Our data also demonstrate that the relative ability of factor IXa and Xa to activate factor VII is significantly different when these reactions occur on tumor cell surfaces as compared with suspensions of mixed phospholipids.

Factor VIIa is a trace vitamin K-dependent glycoprotein that circulates in blood as a precursor to the serine protease, factor VIIa. Single chain human factor VII is converted to two chain factor VIIa by the cleavage of a single internal peptide bond located in the middle of the molecule at Arg\(_{127}\)Ile\(_{133}\). Factor VIIa, in turn, activates either factor X or factor IXa in a reaction that requires a lipoprotein cofactor designated tissue factor.2

A number of studies have been carried out to assess the relative abilities of factors IXa and Xa to activate factor VII. Previous studies with those obtained using the traditional suspension of mixed brain phospholipids. In this report, we demonstrate that these tumor cells support both the factor IXa and factor Xa-activated reactions. Our results demonstrate that the relative ability of factor IXa and factor Xa to activate factor VII is significantly different when these reactions occur on tumor cell surfaces as compared with suspensions of mixed phospholipids.

We have carried out studies to assess the relative abilities of factors IXa and Xa to activate factor VII on a human bladder carcinoma cell line (J82) and compared these rates with those obtained using the traditional suspension of mixed brain phospholipids. In this report, we demonstrate that these tumor cells support both the factor IXa and factor Xa-mediated activation of factor VII and provide kinetic data that factor Xa catalyzes this reaction at a rate roughly sixfold greater than factor IXa. In addition, we provide new rate data on the activation of factor VII by factor IXa and factor Xa in the presence of mixed brain phospholipids and calcium ions.

MATERIALS AND METHODS

Bz-Ile-Glu-Gly-Arg-p-nitroanilide (S-2222) was obtained from Helena Laboratories, Beaumont, TX. Bovine serum albumin (fatty acid free), rabbit brain cephalin, Tris (Trizma base), human high density lipoprotein, human low density lipoprotein, human very low density lipoprotein, and phosphorus standard solution were products of Sigma, St Louis. Highly purified human factor VIII (Monoclate VIIa) was obtained from Armour Pharmaceutical Co, Kankakee, IL. Protein assay dye reagent concentrate, Coomassie Brilliant Blue R-250, and sodium dodecyl sulfate (SDS) were products of Bio-Rad, Richmond, CA. SDS low molecular weight (mol wt) standard kit was purchased from Pharmacia, Piscataway, NJ. Nitrocellulose was obtained from Schleicher and Schuell, Keene, NH. \(^{125}\)I-Protein A (70 to 100 μCi/\(\mu\)g) was obtained from New England Nuclear, Boston. All other reagents were the best grade available from commercial sources.

Normal pooled plasma, assumed to contain 1 U/mL factor VII activity, was prepared by pooling citrated plasma from 20 healthy volunteers. The plasma was stored at -70°C until use. The pH of the plasma samples was adjusted to 7.4 with 1 N NaOH buffer. Assays were performed at 37°C and the absorbance at 405 nm was monitored. The rate of hydrolysis of Bz-Ile-Glu-Gly-Arg-p-nitroanilide (S-2222) was measured at 1 U/mL factor VII activity. The data was consistent with the hypothesis that HUVEC have the equivalent of procoagulant phospholipid. Rao et al\(^2\) have also reported that freeze-thawed platelets can enhance factor VII activation by factor Xa and Ca\(^{2+}\), whereas unstimulated platelets do not support this reaction. Again, these investigators contend that this rate enhancement is due to anionic phospholipids, which becomes available for the reaction on disruption of the platelet membrane.

From the Blood Systems Research Foundation Laboratory, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM.

Submitted September 1, 1988; accepted January 23, 1989.

Supported by research grants from the National Institute of Health (HL 35246) and Blood Systems, Inc.


Address reprint requests to Walter Kisiel, PhD, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

\(1\) 1989 by Grune & Stratton, Inc.
ACTIVATION OF FACTOR VII BY FACTORS IXa, Xa

donors and storing at −80°C. Factor VII-deficient plasma (<1% factor VII antigen) was obtained from a hereditary factor VII-deficient patient. Factor IX and factor VII-deficient plasmas were obtained from hereditary factor IX and factor VIII-deficient patients. Human recombinant factor VIII (des B-domain) and human recombinant tissue factor apoprotein were kindly provided by Drs Dan Eaton and Lisa Paborysky, Genentech Inc, San Francisco. Human brain cephalin (mixed brain phospholipids) was prepared according to the procedure of Bell and Alton.13 The phospholipid concentration in the stock cephalin solution was determined according to the method of Chen et al.14 Protein concentrations were determined by the Coomassie Blue dye binding assay using bovine serum albumin as the reference protein.15

Cell culture techniques. The human bladder carcinoma cell line (J82) was obtained from American Type Culture Collection, Rockville, MD. Cells were maintained at 37°C in an atmosphere containing 6% CO2 and 98% relative humidity. Cells were grown in either T-75 flasks or 12-well plates containing Eagle’s minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories Inc), nonessential amino acids (Mediatech Inc), and penicillin-streptomycin (Sigma). Cells that were used in factor VII activation studies were harvested by an initial wash of 5 mL 0.01 mol/L sodium phosphate/0.15 mol/L NaCl (pH 7.5) followed by a ten-minute incubation with 5 mL 0.4% trypsin (Sigma). Following detachment of the cells, an additional 5 mL of media was added. This mixture was centrifuged (300 g; seven minutes), the supernatant discarded, and the cells resuspended in 10 mL of fresh media. A cell count was obtained and the suspension recenterfuged (300; seven minutes). The supernatant was discarded, and the cells resuspended in 0.05 mol/L Tris-HCl/0.1 mol/L NaCl/0.1% bovine serum albumin (pH 7.5) at a cell density of 5 × 10⁶ cells/10 μL of solution. A 20-μL aliquot of this cell suspension was used directly in the factor VII activation experiments. All J82 cell suspensions used in the activation studies were >98% viable as assessed by the trypan blue exclusion method.

Factor VIII activation. Human factor VIII (Monoclate VIII:c) was activated by incubation at 37°C with human α-thrombin at an enzyme-substrate weight ratio of 1:400. The progress of factor VIII activation by thrombin was assessed in a one stage coagulation assay using factor VIII deficient plasma. At the point of maximal factor VIII activation, aliquots of factor VIIIa were removed from the incubation system and immediately added to the factor IXa-factor VII activation mixtures.

Factor V purification and activation. Factor V was purified from normal pooled human plasma by a method involving polyethylene glycol precipitation and DEAE-Sepharose CL-6B column chromatography essentially as described by Suzuki et al.15 Conversion of factor V to Va was carried out by incubation with the purified factor V activator from Rous sarcoma venom at an enzyme-substrate weight ratio of 1:100 as previously described.18 Factor VII purification. Human factor VII, factor IX, and factor X were partially purified from therapeutic plasmapheresis plasma by a combination of dextran sulfate agarose (DSA) chromatography and Sephadex G-150 column chromatography as previously described.20 Factor X was activated by incubation with an insolubilized preparation of RVV-X as described.21 Final purification of factor Xa was performed by gel filtration on a Sephadex G-100 column (2.6 x 90 cm) equilibrated with 0.05 mol/L Tris-HCl (pH 7.5)/0.1 mol/L NaCl/1mmol/L benzamidine and eluted at a flow rate of 0.5 mL/min. The final product was pure as assessed by SDS-PAGE, and was essentially all factor Xaβ with a small amount of factor Xaa.

Preparation of factors IXaa and IXaβ. The partially purified factor IX pool obtained from DEAE-Sepharose chromatography was further purified by DSA column chromatography as previously described.20 Human IXaa and IXaβ were prepared by incubating factor IX with insolubilized RVV-X followed by gel filtration in Sephacryl S-200 as described for the preparation of bovine factors IXa and IXaβ by Byrne and Castellino.22 Both factor IXα and IXβ were pure as assessed by SDS-PAGE. Under reducing conditions factor IXaβ migrated as two bands with mol wt values of 28,000 and 17,000, whereas factor IXaα migrated as a single broad band exhibiting an apparent mol wt of 28,000 to 30,000. One microgram of our factor IXaβ preparation produced a clotting time of 45 seconds in a system containing 100 μL TBS-BSA, 100 μL rabbit brain cephalin, 100 μL normal pooled plasma, and 100 μL 25 mmol/L CaCl2. In an identical assay, one microgram of our factor IXaa preparation produced a clotting time of 62 seconds. The clotting activities of both factors IXaα and IXaβ were also determined by incubation with nonactivated factor IX deficient plasma as previously described.23 Under these conditions, factor IXαaa had approximately 20% the clotting activity of human factor IXαβ, and is in agreement with the relative clotting activity of human factors IXα and IXβα as reported by Griffith et al.21 In order to demonstrate that each of these preparations were free of contaminating factor Xa, 100 μL of a 1.6 μmol/L solution of either factor IXα or IXβα was incubated with 900 μL of a buffer containing 0.05 mol/L Tris-HCl/0.15 mol/L NaCl/1.0 mmol/L S-2222 (pH 8.3). No change in absorbance at 405 nm was observed in either incubation mixture even after prolonged incubation at 37°C (>30 minutes).

Preparation of antibodies. Rabbit anti-human factor V IgG was kindly provided by Dr William Kane, University of Washington, Seattle. Antibodies against human factor VII and recombinant human tissue factor apoprotein were produced in rabbits following immunization with purified antigens.24 Each antiseraum was mixed with an equal volume of neutral, saturated ammonium sulfate, stirred for one hour at room temperature, and centrifuged. The resulting pellet was redisolved in a minimal volume of 0.05 mol/L Tris-HCl (pH 7.5)/0.1 mol/L NaCl/0.02% NaN3 (TBS/NaN3) dialyzed at 4°C against this buffer, and applied to a Protein A-Sepharose column (2 x 2 cm) equilibrated at room temperature with TBS/NaN3. Following extensive washing with TBS/NaN3, the IgG fraction was eluted with 0.1 mol/L glycine-HCl (pH 2.5)/0.5 mol/L NaCl and fractions collected in 1/10 volume of 1 mol/L Tris-HCl (pH 8.8) to immediately neutralize the acidic eluent. Purified IgG was subsequently dialyzed against TBS/NaN3, at 4°C, and aliquots stored at −80°C. Affinity-purified polyclonal anti-
human factor VII IgG was prepared essentially as described using factor VII-Affi-Gel 15 columns.

**Assay of factor VII activation.** Conversion of factor VII to factor VIIa was measured by monitoring the decrease in clotting time of hereditary factor VII deficient plasma produced by temporal aliquots of each incubation mixture. Activation reactions were performed in 250 μL snap cap polypropylene tubes at 37°C. Activation mixtures consisted of 640 mmol/L factor VII, 5.0 mmol/L CaCl2, 0.5 mmol/L phospholipid, and varying concentrations of either factors IXα, IXαβ, or Xa in a total volume of 100 μL human brain thromboplastin, and 100 μL of 0.05 mol/L Tris·HCl/0.10 mol/L NaCl/0.1% bovine serum albumin, pH 7.5 (TBS/BSA). The phospholipid consisted of either a 1:100 dilution of stock cephalin (0.5 mmol/L, final phospholipid concentration) or 1.0 x 10^6 J82 cells. In control experiments, factor IXα or factor Xa was replaced with an equal volume of TBS-BSA. All reactants were added in the above order, and the reaction was initiated by the addition of either factor IXα or factor Xa. At selected times, 10 μL aliquots were removed from the incubation mixture and added to 190 μL of TBS-BSA containing 6 mmol/L CaCl2. Clotting times were converted to units per milliliter activity from a standard curve constructed with 1:10 to 1:200 dilutions of normal pooled plasma. The conversion of factor VII to VIIa was also monitored by SDS-PAGE and Western blotting. As in the clotting assay, 10 μL aliquots of each incubation mixture were removed at selected intervals and the mixture subjected to SDS-PAGE following reduction with 10% β-mercaptoethanol. Following electrophoresis, proteins were electrophoretically transferred from the gel to nitrocellulose membranes, and factor VII/VIIa visualized by incubation with affinity purified rabbit anti-factor VII IgG and [125I]protein A.

**Activation of factor VII by factor Xa on J82 cells in the presence of anti-tissue factor apoprotein IgG.** Monolayers of J82 cells were grown to confluency on 12 well plates (3.8 cm²/well). Wells were washed three times with 1.5 mL aliquots of 10 mmol/L HEPES/137 mmol/L NaCl/4 mmol/L KCl/11 mmol/L glucose (pH 7.45). Cells were then incubated at 37°C in a solution of 10 mmol/L HEPES/137 mmol/L NaCl/4 mmol/L KCl/11 mmol/L glucose/0.5% BSA/5 mmol/L CaCl2 (pH 7.45) containing either rabbit anti-tissue factor apoprotein IgG (0.8 mg/mL) or pre-immune rabbit IgG (0.8 mg/mL). As revealed in separate experiments, this concentration of anti-tissue factor IgG completely blocked [125I]-factor VII and [125I]-factor VIIa specific binding to the J82 monolayer. After a two-hour incubation, factor VII (320 nmol/L) and factor Xa (5.7 nmol/L) were added to the incubation mixture. Thereafter, at five-minute intervals, 10 μL aliquots were removed and the factor VII clotting activity determined as described earlier.

**SDS-PAGE.** SDS-PAGE was performed according to Laemmli using a 2.5% polyacrylamide concentrating gel and a 10%
polyacrylamide resolving gel. Gels were stained for one hour in 0.05% Coomassie Blue R-250/50% methanol/10% acetic acid and diffusion destained in 10% methanol/10% acetic acid.

RESULTS

Purification of single chain factor VII by mono Q-FPLC chromatography. Affinity purified human factor VII was applied to a Pharmacia Mono-Q column in order to completely separate single chain factor VII from any contaminating factor VIIa. Fractions were divided into three pools based on SDS-PAGE, activity measurements, and Western blots (Fig 1). Pool A consisted of homogenous, single chain factor VII as assessed by SDS-PAGE and Western blotting with a specific activity of 2 to 3 U/μg protein. While pool B factor VII appeared to be pure, single chain factor VII in SDS-gels stained with Coomassie Blue (Fig 1B), it nonetheless contained small amounts of factor VIIa when analyzed by Western blots (Fig 1C). The specific coagulant activity of pool B factor VII was 6 U/μg protein. Pool C factor VII contained 10% to 20% factor VIIa when analyzed by SDS-PAGE and Western blots (Figs 1B and C). Accordingly, all activation experiments described in this report used pool A factor VII.

Activation of factor VII by factors IXα and Xα on J82 cell surfaces. In order to determine whether J82 cells possess procoagulant phospholipid capable of supporting factor Xα and factor IXα-mediated coagulation reactions, we examined the rate of factor VII activation by purified factor Xα and factor IXαβ in a suspension of 1.0 x 10⁶ J82 cells in the presence of 5 mmol/L CaCl₂. The relative ability of factor IXαβ and factor Xα to activate factor VII on the J82 cell surface is illustrated in Fig 2. In these experiments, factor VII (640 nmol/L) that originally had a specific activity of 3 U/μg, reached a specific activity of 20 U/μg after a five-minute incubation period using 1.36 nmol/L factor Xα. A similar rate of activation by factor IXαβ was achieved using a final concentration of factor IXαβ at 8.5 nmol/L. This represents a 6.3-fold molar, or a five-fold weight, difference in the relative abilities of factor Xα and factor IXαβ to activate factor VII. In addition, the relative activation rates of factor VII by factors Xα and IXαβ were unchanged when the reactant concentrations were reduced 50-fold in the presence of 1.0 x 10⁶ J82 cells and 5 mmol/L CaCl₂ (data not shown). When these experiments were repeated in the absence of cells, no activation of factor VII by either factors IXα or Xα was observed. Furthermore, no microscopic evidence for J82 cell fragmentation or lysis was observed after incubation with the clotting factors. Since it has previously been postulated that factor Va may serve as a high affinity binding site for factor Xα on endothelial cell surfaces,29 we examined whether factor Va was capable of altering the rate of factor VII activation by factor Xα on J82 cell surfaces. We found that the addition of stoichiometric amounts of exogenous human factor Va had no measurable effect on the activation rate of factor VII by factor Xα. In addition, pretreatment of J82 cells with rabbit anti-human factor V IgG was without effect on the activation of factor VII by factor Xα. These results are similar to those recently reported for the activation of factor VII by factors Xα and Va on activated platelets12 as well as human umbilical vein endothelial cells.11

Since J82 cells are known to produce relatively large amounts of cell surface tissue factor, we also examined whether the observed factor VII activation was associated with factor VII binding to tissue factor and subsequent activation by factor Xα. To test this, we incubated confluent J82 monolayers with either rabbit anti-tissue factor apoprotein IgG or pre-immune rabbit IgG. The results of these experiments revealed that a two-hour preincubation of the cell surface with neutralizing anti-tissue factor IgG (0.8 mg/mL) had no effect on the activation rate of factor VII (320 nmol/L) by factor Xα (5.7 nmol/L).

Factor VII activation by factors Xα and IXα on mixed brain phospholipid micelles. The ability of purified factors Xα and IXαβ to separately activate factor VII in the presence of 5 mmol/L Ca++ and mixed brain phospholipids (0.5 mmol/L) is shown in Fig 3. Factor VII (640 nmol/L) with a starting specific activity of 3 U/μg, reached a specific activity of 20 U/μg after a four-minute incubation with 0.23 nmol/L factor Xα. In order to achieve a similar rate of factor VII activation by factor IXαβ, a 4.27 nmol/L solution of factor IXαβ was required. These results indicate an 18.5-fold molar difference, or a 15-fold weight difference, in the ability of factor Xα to activate factor VII as compared with the activation of factor VII by factor IXαβ. These findings were very reproducible in repeated experiments using different protein preparations. In addition, reducing the phospholipid concentration to 0.05 mmol/L had no significant effect on
the relative potencies of factors IXa and Xa with respect to factor VII activation. Moreover, as was observed for the J82 cell suspension, the relative activation rates of factor VII by factors Xa and IXa remained unchanged when the reactant concentrations were reduced 50-fold. This data is significantly different from the 800-fold difference in the relative efficiency of factor IXa and factor Xa to activate factor VII reported by Masys et al. When the activation experiments were performed in the absence of added phospholipid, no activation of factor VII by either factors IXa or Xa was observed. In addition, no activation of factor VII by factors IXa and Xa was observed when the mixed brain phospholipids were replaced with either VLDL (0.1 mg/mL), LDL (0.5 mg/mL), or HDL (1.0 mg/mL). Since the relative difference in the activation rate of factor VII by factors IXa and Xa in the presence of phospholipid and Ca2+ was markedly less than previously reported, the question arose of possible contamination of our factor IXa preparation with factor Xa. In order to fully eliminate this possibility, we incubated our factor IXa preparation with the chromogenic substrate, S-2222. This substrate is specific for factor Xa and is not cleaved by factor IXa. As expected, no change in absorbance at 405 nm was observed when S-2222 was incubated with our factor IXa preparations (data not shown).

In addition to factor VII activation by factor IXa, we also examined the ability of factor IXa to activate factor VII. As shown in Fig 4, factor IXa (3.57 nmol/L) activated factor VII (640 nmol/L) at a rate approximately half that observed...
When factor VII was activated by factor IXαβ (4.27 nmol/L). This data was somewhat surprising since our factor IXα preparation exhibited only 20% of the clotting activity observed for factor IXαβ. Although not examined further, conceivably, small amounts of factor IXαβ could be converted to factor IXαβ in the incubation mixture by catalytic amounts of factor VIIa in complex with either cell-surface tissue factor or phospholipid, thus accelerating the apparent rate of factor IXα catalysis in this purified system in comparison to the clotting assay. In an effort to determine whether the high molecular weight cofactors that accelerate the activation of factor X and prothrombin also augment the rate of factor VII activation by factor IXα and factor Xα, we added purified factor Vα and factor VIIα/VIIa to mixtures of factor VII containing factors Xα and IXα, respectively. The addition of stoichiometric amounts of purified factor Vα to the factor Xα-Ca2+-phospholipid mixture had no effect on the rate of factor VII activation by factor Xα (data not shown). Likewise incubation of factors IXα and IXαβ with either recombinant factor VIII (des B-domain) or thrombin-activated factor VIII, in the presence of Ca2+ and mixed brain phospholipid, failed to augment the rate of factor VII activation by these proteases.

Analysis of factor VII activation by Western blotting. In order to compare and analyze the activation and potential degradation products generated by the factors Xα and IXα-catalyzed activation of factor VII, incubation mixtures were subjected to Western blotting as well as simultaneous clotting activity measurements. As shown in Fig 3B and C, no differences were observed in the factor VII activation products generated by either factor IXαβ or factor Xα. In our activation system, single-chain zymogen factor VII, factor VIIα heavy chain, and factor VIIα light chain were visible. No factor VII-VIIα degradation products were observed in either of the two activation mixtures.

DISCUSSION

We have conducted experiments to assess the relative activation rates of human factor VII by human factor IXα and factor Xα on J82 cell surfaces as well as on mixed brain phospholipids. Our data clearly indicate that J82 cells possess procoagulant phospholipid capable of supporting the activation of factor VII by either factor IXα or factor Xα. Furthermore, our experiments demonstrate that the relative ability of factor IXα and factor Xα to activate factor VII is significantly different when activation occurs on J82 cell surfaces in comparison with a suspension of mixed brain phospholipids. Under our experimental conditions, factor Xα was sixfold more efficient than factor IXαβ at catalyzing the activation of factor VII when activation takes place in a suspension of tumor cells. On the other hand, when factor VII activation was examined in the presence of mixed brain phospholipids, we found that factor Xα was roughly 18-fold more efficient than factor IXαβ at activating factor VII. This finding was somewhat surprising as an earlier study10 had reported that factor Xα was 800-fold more efficient than factor IXαβ at activating factor VII under similar conditions. When first presented with this striking difference, we considered the unlikely possibility that factor Xα was contaminating our factor IXα preparations. Clearly this was not the case since our factor IXα samples were incapable of cleaving the factor Xα-specific chromogenic substrate, S-2222. Our data were further substantiated when we activated factor VII using one of the RVV-X activation products of factor IXα known as factor IXαβ. In that study, factor IXαβ catalyzed the activation of factor VII at a relative rate one half of that observed when factor VII is activated by factor IXαβ. Precisely why the relative activation rate values obtained in this study using mixed brain phospholipids are vastly different than earlier values is unknown. Perhaps crucial in this regard are potential differences in specific coagulant activities of each protease preparation used in each study. It is interesting to note, however, that our relative activation rates are very similar to the 20-fold difference in activation rates obtained by Masys et al10 in incubation mixtures without added phospholipid. Concurrent with activity measurements, we subjected temporal aliquots of factor VII activation mixtures to SDS-PAGE and subsequent Western blotting in order to determine whether additional cleavages occurred in factor VII beyond the activation cleavage at Arg154Lys155. Western blots of each activation mixture demonstrated that the factor VII cleavage products generated by either factor Xα or factor IXαβ were apparently identical (Fig 3B and C). In each system, single chain factor VII (mol wt 50,000) was converted to a two chain factor VIIα consisting of a heavy chain (mol wt 34,000) and a light chain (mol wt 28,000). No additional cleavages were observed in Western blots of reduced SDS-gel samples from each activation reaction, and is consistent with previous reports describing the activation of human factor VII.20,32

Inasmuch as the proteolytic activity of factor Xα and factor IXα toward prothrombin and factor X, respectively, is augmented several orders of magnitude, we examined whether these high molecular weight cofactors had any effect on their respective protease with regard to factor VII activation. In this regard, we found that neither human factor VIII/VIIα nor purified human factor Vα had any measurable effect on the activation of factor VII by factors IXαβ and Xα, respectively. This is in accord with recent reports describing the activation of factor VII by factor Xα on endothelial cells and platelets in the presence of exogenous factor Vα.11,12 In addition, based on our activation experiments involving preincubation of J82 cells with anti-tissue factor apoprotein IgG, factor VII activation on J82 cells appeared to be independent of cell surface tissue factor in the time frame of our incubation.33,34

Our results indicate that J82 tumor cells possess procoagulant phospholipid that readily supports the activation of factor VII by factors IXα and Xα. This procoagulant phospholipid appears to be similar, if not identical, to the coagulant-active phospholipid that supports the activation of factor VII by factor Xα on freeze-thawed platelets12 and endothelial cells.11 Our findings suggest a mechanism for the activation of factor VII on solid tumors that ultimately leads to fibrin deposition in the connective tissue surrounding

From www.bloodjournal.org by guest on October 30, 2017. For personal use only.
viable tumor cells.39 Whether coagulation activation and fibrin deposition on and around tumor cells contributes to the progression of human malignancy in all cases is uncertain, although certain types of neoplasms such as small cell carcinoma of the lung (SCCL) and non-SCCL are sensitive to anticoagulant therapy using either warfarin or dipiridamole derivatives.36,37 Our data also provides evidence that the J82 tumor cell surface phospholipid may play a role in the relative ability of factor IXa and Xa to activate factor VII in comparison to mixed anionic phospholipid micelles ordinarily used in in vitro activation experiments. Other possibilities, however, such as the presence of serine protease inhibitors on the J82 cell surface that preferentially neutralize factor Xa, may also be responsible in part for this effect and need to be investigated further. Last, our data strongly suggest that factor IXa may play a much greater role in factor VII activation in vivo than previously recognized.

ACKNOWLEDGMENT

The authors are grateful to Drs Dan Eaton and Lisa Paborsky of Genentech, Inc for providing us with preparations of recombinant factor VIII and tissue factor apoprotein. In addition, thanks are due to Drs William Kane and Masashi Noguchi for providing preparations of anti-factor V IgG and factor Va, respectively, and Dr Toshiyuki Sakai for helpful discussions. The human therapeutic plasmapheresis plasma used in this study was generously provided by United Blood Services of Albuquerque.

REFERENCES


Activation of human factor VII by factors IXa and Xa on human bladder carcinoma cells

P Wildgoose and W Kisiel

Updated information and services can be found at: http://www.bloodjournal.org/content/73/7/1888.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml