Cooperative Interaction between Factor VII and Cell Surface-expressed Tissue Factor*

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Assembly of the extrinsic pathway on cell surfaces was investigated by studying the binding and activity of factor VII on the bladder carcinoma cell line J82 which expressed 18,800 milliunits of tissue factor activity/10^6 cells. In binding studies, the association of factor VII to monolayers of cells was time-, temperature-, and calcium-dependent. The ligand binding was specific, reversible, and saturable. This interaction was inhibited by a monoclonal antibody to human brain tissue factor. Factor VII added to the cells was recovered as factor VII rather than factor VIIa when incubated in the presence of factor X neutralizing antibodies, suggesting that these cells produced factor X. Specific factor VII binding to the cell revealed a sigmoidal binding isotherm with half-maximal binding occurring at 314 ± 145 pm to 38,300 ± 14,300 sites/cell. Hill plots of the binding data indicated an average slope of 2.1. Binding parameters were also determined kinetically. At maximal factor VII:factor VII complex formation the apparent K_a for factor X was 274 nM, the V_max was 4.15 nM/min, and the K_m was estimated to be 14 s^-1. In the presence of excess tissue factor and factor X, increasing amounts of factor VII added to the J82 cells demonstrated a sigmoidal relationship with the rate of factor Xa formation. Hill plots indicated a slope of 2.0 at the lower factor VII concentrations which changed to 1.0 at the higher input amounts of factor VII. Hanes plots were used to determine the apparent dissociation constant of the interaction (222 ± 85 pm). The V_max was 5.54 ± 1.04 nM/min for the cleavage of factor X. These data are consistent with factor VII binding to at least two sites on tissue factor (receptor) with positive cooperativity. Because at saturation the stoichiometry of the factor VII:tissue factor complex is 1:1, tissue factor must be expressed as a dimer on the surface of the J82 cells.

The initiation of coagulation through the extrinsic pathway requires the assembly of factor VII and tissue factor on a membrane surface in the presence of calcium (1). Tissue factor is a nonproteolytic integral membrane glycoprotein cofactor of about 45,000 daltons (2–5). Human factor VII is a vitamin K-dependent single chain glycoprotein of M_r 50,000 (6, 7) and is an active zymogen when associated with tissue factor (8).

Cleavage of factor VII to factor VIIa, a serine protease composed of two disulfide bond-linked chains of M_r 28,000 and 23,000, is associated with a 25–40-fold enhancement in activity (6, 7, 9). The activity of factor VIIa within this assembled macromolecular complex greatly accelerates the cleavage of its substrates, factors IX and X (10), over that observed for the enzyme alone (11, 12). Recently, it has been suggested that there is an ordered addition of the enzyme to the cofactor before binding and catalysis of the substrate (13) and that the rate of factor Xa formation is controlled by the free substrate concentration (14).

Regulation of procoagulant events may be mediated through the expression of tissue factor on cell surfaces during hemostatic reactions intravascularly or at extravascular sites of inflammation. The interaction of factor VII and tissue factor has been studied using nonvascular cells that constitutively synthesize this cofactor (15–18), as unstimulated vascular cells do not express procoagulant activity (19–21). A mechanism to initiate coagulation at specific vascular and extravascular sites may be controlled by induced expression of tissue factor by monocytes (22, 23) and endothelial cells (19–21). Factor VII can be synthesized by induced monocytes (24) and macrophages (25) indicating that the components required for the initiation of coagulation by the extrinsic pathway can be produced locally by these effector cells. Tissue factor activity can be expressed on cultured tumor cells or vesicles from them as measured by factor Xa formation in the presence of added purified factor VII (26–28). This activity could be neutralized by specific antibody to tissue factor (26).

Tumor cell surfaces have also been shown to support the later stages of blood coagulation (29), suggesting that hemostasis may play a central role in tumor pathogenesis (30). However, no details describing the binding reactions of factor VII and functional output of the assembled extrinsic activation complex on the surface of tumor cells expressing high concentrations of tissue factor have been documented. In this report, we describe the binding of factor VII to cell surface-expressed tissue factor as a receptor-mediated process. Direct binding assays and activity analysis of the formed extrinsic activation complex indicate that factor VII binds with positive cooperativity to at least two sites on tissue factor.

MATERIALS AND METHODS

Materials—Tissue culture flasks and 6-, 12-, 24-, and 96-well plates were purchased from Costar. Fetal bovine serum was bought from Irvine Scientific. All other tissue culture reagents were from Flow Laboratories. Na^131I was obtained from Du Pont-New England Nuclear. Enzyme-treated radiiodination reagent and polyacrylamide gel reagents were purchased from Bio-Rad. Hoescht dye 33258 was
obtained from Behring Diagnostics, and rabbit brain thromboplastin was purchased from Difco. The chromogenic substrate bought from Helena Laboratories. All other reagents were of the highest purity available and were obtained from Sigma.

**Cell Culture** — The human bladder cancer carcinoma J82 cell line was obtained from the American Type Culture Collection (ATCC, HTB 1). Cells were maintained in DMEM supplemented with 10% fetal bovine serum, penicillin, streptomycin, and L-glutamine, at 37 °C in a humidified 10% CO2 environment. Cultures were subcultured using phosphate-buffered saline (10 mM phosphate, 150 mM NaCl, pH 7.4) supplemented with 1 mM EDTA, diluted in culture medium, and plated in fresh flasks or in 12- or 24-well flat-bottom tissue culture plates. All assays were conducted within 24 h of confluence.

Quantification of cell number was determined by fluorometric determination of DNA content as described by Downs and Wilfinger (31) using salmon sperm DNA as the standard. The concentration of DNA correlated directly with cell number (between 106 and 108 cells).

**Procoagulant Activity** — Cells were washed in 10 mM Hepes, 150 mM NaCl, pH 7.4 (HBS) and disrupted in HBS containing 15 mM octyl glucopyranoside as described previously (24). Quantitation of tissue factor activity was determined from a standard curve generated from serial dilutions of rabbit brain thromboplastin in recalcified plateletpoor human plasma. To 100 μl of human plasma was added 100 μl of cell lysate and following incubation at 37 °C for 1 min, coagulation was initiated by the addition of 100 μl of prewarmed 25 mM CaCl2. A clotting time of 50 s was defined as 1 unit/ml tissue factor activity.

**Proteins** — Human factors VII (32) and X (33) were isolated from plasma as previously described. Both products were homogenous as judged by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in the presence and absence of a reducing agent (34). The IgG fraction of rabbit anti-human factor X was previously described (33). A monoclonal antibody (IgG1, α) to human brain tissue factor VII was prepared according to the basic method of Kohler and Milstein (35) and was selected for its ability to recognize fluid-phase factor VII. Culture supernatants had the capability to neutralize factor VII activity following incubation with purified factor VII for 1 h at 37 °C and evaluation in an one-stage factor VII assay. A neutralizing monoclonal antibody (IgG4, α) to human brain tissue factor was a gift from Dr. James H. Morrissey (Research Institute of Scripps Clinic, La Jolla CA).

**Radioiodination** — Factor VII (50 μg) was labeled with Na125I (0.5 mCi) to a specific activity of 0.42–1.47 μCi/μg by the coupled factor-peroxidase/glucose oxidase method (Enzymobead radioiodination reagent) using 25 μl of the rehydrated reagent at room temperature for 10 min. Iodinated protein was separated from free iodine by gel filtration over a 12-mL Sephadex G-25 column equilibrated in phosphate-buffered saline containing 1% bovine serum albumin (BSA). The iodinated labeled molecule retained about 100% of its functional activity, as assessed in an one-stage factor VII clotting assay (32).

**Binding Assays** — The methodology of Stern et al. (36) was used to measure the association of 125I-factor VII to J82 cells. Twelve-well Costar plates were seeded with 1–5 × 104 cells/well, and confluent cultures were washed three times with buffer A (10 mM Hepes, 137 mM NaCl, 4 mM KCl, and 11 mM glucose, pH 7.45). Duplicate or triplicate wells were incubated with up to 50 ng/ml (1 nM) 125I-factor VII in buffer A supplemented with 0.5% BSA and 5 mM CaCl2 (buffer B) and incubated for 2 h at 37 °C with constant oscillation (50 rpm) on a model 02 gyratory shaker (New Brunswick Scientific Co.). After this period, the plates were rapidly washed six times with cold buffer B (6 mM Tris/HCl, 140 mM NaCl, and 0.5% BSA, pH 7.45). Specific factor VII binding was determined by subtracting the labeled factor VII associated with the cells in the presence of 50-fold molar excess of unlabeled factor VII from the total cell-bound 125I-factor VII. Cells associated with factor VII was determined after lysing the cells in 1 ml of 200 mM NaOH, 1% SDS, and 10 mM EDTA and counting in an Iso Data 20/20 series γ counter.

**Inhibition Studies** — Inhibition of factor VII binding to J82 cells by a monoclonal antibody to tissue factor was determined after wells of a 24-well tissue culture plate were seeded with 10000 J82 cells. The wells were washed three times with buffer A, and then triplicate wells were incubated with 1 μl of buffer A+ or buffer A+ containing either 0.1 mg of normal mouse IgG or specific antibody for 2 h at 37 °C. Wells were washed twice with buffer A+, incubated with 125I-factor VII (50 ng/ml in buffer A+) for 2 h at 37 °C, washed six times with cold buffer B, lysed, and bound radioactivity measured.

Prevention of cleavage of 125I-factor VII during the 2-h incubation period was determined in the presence of several inhibitors. Duplicate wells containing 125I-factor VII (50 ng/ml) or ligand supplemented with 2 mM diisopropyl fluorophosphate, 1/10 dilution of a culture supernatant from a hybridoma cell line capable of neutralizing factor VII activity, or 1 mg/ml of the IgG fraction of normal or anti-factor X rabbit serum were incubated for 2 h at 37 °C. The wells were washed and processed as described above except the cells were dissolved in SDS running buffer, electrophoresed in SDS-9% polyacrylamide gels under reducing conditions, and developed by autoradiography (34).

**Functional Assays** — The rate of factor X activation was used as a kinetic approach to monitor factor VII binding. Each well of a 24-well Costar plate received 2 × 104 J82 cells. At confluency, varying concentrations of factor VII (0.5–80 ng/ml) were added to duplicate wells and incubated for 2 h at 37 °C. The cultures were washed rapidly three times with buffer B and once with buffer A+. Each well received 0.7 ml of 50 μg/ml factor X in buffer A+ and was incubated at ambient temperature. At 3, 6, 9, 12, 15, 25, and 30 min, 50 μl aliquots were removed and added to individual wells in a 96-well U-bottom plate containing 25 μl of 0.4 M EDTA, pH 7.5. After 25 min, each well received 50 μl of 20 mM Tris/ HCl, 150 mM NaCl, 0.5% BSA (pH 8.3) follow by 25 μl of 8-fold concentrated chromogenic buffer (400 mM Tris/HCl, 1.8 M NaCl, and 400 mM EDTA, pH 8.3). A factor Xa standard curve ranging from 15.6 to 8000 ng/ml was assayed in duplicate in the same plate. With addition of 50 μl of 1.35 mM S-2222, a change in absorbance at 405 nm was monitored in a microtiter plate reader every 90 s. The data were collected for 15–20 min and analyzed using an IBM PC employing Lotus 1-2-3. From plots of the initial rates of S-2222 cleavage a factor Xa standard curve was constructed and used to determine the amount of factor Xa present at each experimental time point. The rate of factor Xa formation was then calculated for each concentration of factor VII added to the cells.

**RESULTS**

In preliminary experiments the J82 cells were assayed for tissue factor activity along with 16 other established tumor cell lines (data not shown). The J82 cells were found to contain the highest concentration of procoagulant activity equivalent to 18,800 millionites/106 cells. These cells were selected as a model to investigate the association of factor VII with the surface of tumor cells expressing tissue factor.

The binding of 125I-factor VII was first investigated for time, temperature, and calcium dependence. Cells were incubated with 50 ng/ml 125I-factor VII at 4 and 37 °C, and at varying times, the cells were washed and the bound radioactivity measured. Binding was shown to be both time- and temperature-dependent and reached an apparent steady state after 2 h (Fig. 1). About three times more factor VII was associated with the J82 cells at 37 than at 4 °C. When binding experiments were carried out for longer times, no change in the amount of factor VII bound to the cells was observed suggesting that the ligand was not being internalized. Factor VII association to the J82 cells was next studied as a function of calcium concentration. When incubation in final calcium concentrations from 1 to 2 mM a significant increase in 125I-factor VII binding was seen (Fig. 2). Maximal binding was achieved at 4 mM and remained stable to 20 mM of added cation. For the remaining study we selected binding for 2 h at 37 °C in 5 mM calcium as constant parameters.

The specificity of binding was studied by incubating the cells with 1 nM 125I-factor VII and a 100-fold molar excess of unlabeled factor VII, other coagulation proteins, or unrelated molecules. As shown in Table 1, only the homologous competitor (unlabeled factor VII) inhibited ligand binding to about 50%. Other vitamin K-dependent or unrelated proteins did not interfere with 125I-factor VII binding to the cells. It was noted that both factors IX and X appeared to enhance

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1 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate.
The means and standard deviations from seven experiments are given, and the percent bound is normalized to the buffer control.

| Competitor | I125-Factor VII bound (fmol/well) | Bound (%) |
|------------|---------------------------------|-----------|
| None       | 20.4 ± 1.83                     | 100       |
| Factor VII | 10.7 ± 2.29                     | 53        |
| Prothrombin| 19.0 ± 1.37                     | 94        |
| Factor IX  | 21.4 ± 1.63                     | 105       |
| Factor X   | 23.5 ± 2.65                     | 116       |
| Anthrombin III | 17.5 ± 1.59             | 86        |
| Plasminogen| 19.3 ± 1.75                     | 95        |
| Ovaibumin  | 18.9 ± 3.14                     | 93        |
| Carbone anhydrase | 19.0 ± 2.09       | 94        |
| Factor B   | 18.0 ± 2.27                     | 89        |
| Myoglobin  | 19.0 ± 1.53                     | 98        |
| Transferrin| 19.7 ± 2.27                     | 97        |

The ligand was incubated with the cells for 2 h. A 60-fold molar excess of unlabeled factor VII was added at varying times up to two additional hours and the cell-associated radioactivity measured (Fig. 3). Displacement of the bound ligand was linear for an hour at which time approximately 50% of the ligand was removed. This long half-life suggested a slow dissociation rate for factor VII. About 80% of the bound I125-factor VII could be displaced after 4 min of incubation in the presence of 10 mM EDTA. Further incubation with the chelating agent caused cellular detachment from the plastic precluding the determination of complete reversibility. Thus, the ligand-cell interaction was reversible and divalent cation-dependent.

We next determined if the association of I125-factor VII to the J82 cells was tissue factor-mediated. Cell monolayers were pretreated with a specific monoclonal antibody to human brain tissue factor, normal mouse IgG, or culture medium for 2 h at 37 °C, washed, and I125-factor VII (50 ng/ml) was added for an additional 2 h at 37 °C. After washing, the bound radioactivity was measured. Under these conditions, the ligand binding was inhibited 68% or 62% by the monoclonal antibody to tissue factor relative to normal IgG or buffer control, respectively, indicating that the factor VII was binding to tissue factor.

To resolve if the ligand bound to the cells represented factor VII, we compared the bound ligand extracted from the cells after 2 h of incubation in the absence or presence of selected inhibitors and analyzed the material on SDS-9% polyacrylamide gels under reducing conditions followed by autoradiography (Fig. 4). In the absence of inhibitors the single-chain I125-factor VII was converted to the two-chain activated form. Addition of 2 mM diisopropyl fluorophosphate prevented this cleavage, as did monospecific polyclonal antibodies directed to human factor X. However, a monoclonal antibody capable of neutralizing factor VII activity failed to prevent the cleavage of I125-factor VII. These data suggest that factor X may be produced by the cells, as our antibodies do not cross-react with bovine factor X (37), a component of the culture medium. A unique protease capable of activating factor X may also be expressed by the cells. Thus, the I125-factor VII added to the cells can be recovered from them following incubation under the appropriate conditions.

To determine if the binding of factor VII to the J82 cells was saturable we added increasing amounts of I125-factor VII to confluent cells. Specific binding was assessed by subtracting the ligand bound in a 50-fold molar excess of unlabeled

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Cooperative Interaction of Factor VII and Tissue Factor

Specific binding of factor VII to J82 cells is saturable. Increasing concentrations of ¹²⁵I-factor VII were incubated with confluent J82 cells for 2 h at 37 °C. Specific binding was determined by subtracting the bound radioactivity present in 50-fold molar excess of unlabeled factor VII present in parallel wells from the total bound. Factor VII from the total bound. With increasing amounts of ¹²⁵I-factor VII the binding of the ligand to the cells revealed a sigmoidal binding isotherm (Fig. 5). This was observed in five of six experiments performed. Hill plots of these data indicated an average slope of 2.1, suggesting that factor VII was binding to a minimum of two sites on tissue factor. The binding parameters from five experiments were estimated by:

- 1) half-maximal binding, suggesting an apparent dissociation constant of 314 ± 145 pM; and
- 2) double reciprocal plots of the data points at near saturation concentrations, suggesting an apparent dissociation constant of 539 ± 187 pM which occurred at 38,300 ± 14,300 sites/cell.

As an alternative approach to measuring the binding parameters for the factor VII-tissue factor interaction, we used the functional output of this assembled complex in a kinetic study. To establish optimal reaction conditions of this interaction, we titrated the rate of factor Xa generation as a function of cell number. Varying numbers of J82 cells were seeded in 24-well Costar plates and incubated with 1 nM of factor VII for 2 h at 37 °C in the presence of 5 mM calcium ion. After washing, the rate of factor Xa formation was determined using an amidolytic assay for the enzyme. Fig. 6 depicts a linear relationship between the rate of factor Xa formation and cell number up to 100,000 cells/well. Saturation was reached by 200,000 cells and above (data not shown). Thus, we seeded the wells with 2 × 10⁶ cells to be in tissue factor excess for the kinetic study.

We then determined the affinity of the substrate, factor X, for the macromolecular complex by titrating factor X with respect to the rate of factor Xa formation. Wells seeded with 2 × 10⁶ cells received 1 nM factor VII for 2 h at 37 °C. After washing, varying amounts of factor X were added, and the rate of factor Xa generation was determined (Fig. 7). With increasing amounts of factor X added there was a hyperbolic increase in the velocity of substrate hydrolyzed which approached a maximum around 50 μg/ml added factor X. From double reciprocal plots the Kₘ was estimated to be 274 nM and the Vₘₐₓ was about 4.15 nM/min. From direct binding studies, an input of 1 nM factor VII in 24-well plates resulted...
in 0.343 ± 0.106 ng of specifically bound factor VII suggesting an estimated $k_{on}$ of this reaction to be 14.0 s$^{-1}$. We selected 50 μg/ml factor X as an optimal nonlimiting substrate concentration to use in our study.

The binding parameters of the factor VII-tissue factor interaction were measured in saturation experiments. Factor VII was added in increasing concentrations to the cells and incubated for 2 h at 37 °C. After washing, the rate of factor Xa formation was determined (Fig. 8). A sigmoidal relationship was observed between the amounts of factor VII added and the rate of factor Xa generation. Fig. 9 depicts these kinetic data transformed and analyzed using Hill and Hanes plots (38). Hill plots (Fig. 9A) revealed two slopes; at the lower concentrations of added factor VII the slope was $1.96 ± 0.29$, and at higher input factor VII concentrations the slope changed to $1.02 ± 0.06$. The two regression lines intersect at an input concentration of factor VII of $117 ± 28.8$ pM. The cooperativity index calculated from these experiments ranged from 20 to 26, suggesting positive cooperativity (39). Similar conclusions were reached when the data were graphed on Hanes plots (Fig. 9B). As the concentration of factor VII added decreased, the ratio of factor VII concentration to the rate of factor Xa generation. The concentration of factor VII and the rate of factor Xa generation. The concentration of factor VII and the rate of factor Xa generation.

**DISCUSSION**

Factor VII binding to tissue factor expressed by the J82 tumor cell line is a receptor-mediated process. This interaction was shown to be time-, temperature-, and calcium-dependent. The rapid displacement of $^{125}$I-factor VII from the cells by EDTA further supports the role of divalent cations in this association. No evidence for endocytosis was observed at 37 °C, which agrees with the studies of Broze (23) and Ploplis et al. (17) and is in contrast to the report of Rodgers et al. (18). This difference may be attributed to the different cell types examined. Cellular binding of factor VII was specifically inhibited by a monoclonal antibody to human tissue factor, indicating tissue factor as the cellular receptor for factor VII. The association was specific for factor VII, as only excess quantities of unlabeled factor VII competed for its binding. About 50% of the bound radioactivity was not competed, and this relatively high background appears to be a common property of adherent cells with factor VIIa and fibrinogen binding (17, 40). The interaction was also reversible with 50% of the ligand displaced in about 1 h, indicating a slow dissociation rate; this was similar to the findings for fetal lung cells (18) but slower than the 15 min reported for monocytes (23) or another fetal lung fibroblast (17).

Recovery of $^{125}$I-factor VII as either a single- or two-chain molecule was observed depending on the presence or absence of specific inhibitors. Because the antibodies to human factor X do not cross-react with bovine factor X (37) and because they could prevent the cleavage of factor VII, the J82 cells may have the capacity to synthesize factor X which could participate in the processing of the zymogen factor VII into its activated form. Alternatively, the failure of a neutralizing monoclonal antibody to factor VII suggests that a protease other than factor VII/VIIa may be responsible for activating factor X. These possibilities are under further investigation.

The binding of factor VII to the J82 cells was saturable, but the binding isotherm was sigmoidal and not hyperbolic as
reported for other nonvascular cell types (17, 18). Using direct binding assays the apparent \( K_d \) estimated from half-maximal binding was 314 pm compared to 559 pm calculated from double-reciprocal plots. The former estimate would seem to be a better approximation as the latter assumes a hyperbolic binding isotherm. Our estimate of the apparent \( K_d \) is in general agreement with those determined for other cell lines ranging from 82 to 400 pm (17, 18, 23). Further, the \( K_d \) determined by equilibrium measurements was similar to the values calculated from our kinetic determinations (\( K_d = 222 \) pm) and those employing partially purified human brain tissue factor (\( K_d = 460 \) pm (41)). In contrast, equilibrium measurements conducted with tissue factor reconstituted into defined phospholipid vesicles for factor VII binding were about 100-fold greater (42) than those reported for intact cells (Refs. 17, 18, 23, and this study) or crude tissue factor preparations (41). This may be due to an altered tissue factor resulting from purification or a suboptimal environment around the receptor within the artificial membrane. However, binding parameters of factor VII association with tissue factor-containing vesicles measured kinetically were similar to those reported for the intact cells (13).

The number of factor VII receptors on the J82 cells was estimated to be 38,000, about 10-fold greater than those reported for monocytes (23), but intermediate to the number of receptors expressed by several different cell types (17, 18). Although it has been suggested that the number of factor VII receptors correlates with the ability of cultured cells to initiate coagulation (18), this study and another (17) do not appear to support this hypothesis.

Composition and integrity of the cell surface environment play important roles in controlling the reaction rate of the factor VII-tissue factor activation of factor X. Forman and Nemerson (14) used defined phospholipid vesicles containing 30% phosphatidylserine to demonstrate that the rate of factor Xa formation increased as the free factor X concentration increased. These negatively charged vesicles decreased the apparent \( K_m \) of the complex for factor X by 90% to about 40–60 nM (14). This value is 5-fold lower than the apparent \( K_m \) for factor X determined with J82 cells (274 nM) and with crude tissue factor preparations (205 nM (41)). It may be implied from these data that less than 30% of the phospholipids exposed on the surface of J82 tumor cells or in brain thromboplastin is negatively charged. Further experiments are required to determine the content of negatively charged phospholipid molecules within the J82 cell membranes. The \( k_{on} \) of the assembled tissue factor-factor VII complex for factor X was determined at 14 s\(^{-1}\) for the J82 cells and agrees with the calculated value of 14.1 s\(^{-1}\) from the data for monocytes (23). Therefore, the integrity of the cell is important for optimal substrate conversion as lower \( k_{on} \) values have been reported for the extrinsic complex on reconstituted vesicles (5–6 s\(^{-1}\) (14)) or in crude tissue factor preparations (1.1 s\(^{-1}\) (41)).

We observed the sigmoidal binding curve in five of the six equilibrium binding experiments performed, and Hill plots of the data revealed a single slope of 2.1 suggesting positive cooperativity with a minimum of two binding sites on tissue factor for factor VII. A positive cooperative interaction was suggested by Broze, as double-reciprocal plots of the binding of factor VII to lipopolysaccharide-stimulated monocytes at 37 °C were curvilinear (23). Our finding was further supported by our kinetic experiments which also demonstrated a sigmoidal relationship between the rate of factor Xa formation and the added concentration of factor VII. Transformation of these data indicated that at least two sites on tissue factor were involved with the binding of factor VII at low ligand concentrations and that the interaction showed positive cooperativity. The change in slopes would be consistent with a reduction in the dissociation binding constant for the second ligand relative to the first. Such a finding has been reported for factor VII binding to tissue factor in phosphatidylserine-containing vesicles when an Adair two-site cooperative model was applied to determine the binding parameters (42). These same investigators showed that the phosphatidylserine composition in the reconstituted vesicles was responsible for mediating the induced positive cooperativity for the binding of factor VII to purified tissue factor, as neutral phosphatidylcholine vesicles did not display this phenomenon (42). Our data imply that the surfaces of J82 cells and perhaps activated monocytes (23) contain sufficient quantities of negatively charged phospholipids to permit a similar cell surface organization of the factor VII receptor as defined by the phosphatidylserine vesicles.

Tissue factor expressed on the surface of cells or in reconstituted vesicles binds factor VII with positive cooperativity to at least two sites. At saturating concentrations of factor VII/VIIa, the stoichiometry of factor VII to tissue factor is 1:1, which led to the hypothesis that tissue factor is present on the vesicle surface as a dimer (42). Our data support and extend this hypothesis to cells expressing tissue factor where the receptor exists as a dimer with each subunit containing a single factor VII binding site. Association of factor VII to the first site would perturb the receptor permitting easier binding and full expression of the second ligand as depicted in Fig. 10. Thus, regulation of the extrinsic activation pathway of blood coagulation on cell surfaces differs from other described enzyme-cocatalyst interactions as multiple subunits of cofactor control the assembly and function of this macromolecular enzyme complex. Further, constitutive expression of the receptor (tissue factor) for factor VII by tumor cells in conjunction with increased microvascular permeability (43) may play a fundamental role in tumor survival in vivo by initiating coagulation which culminates in fibrin deposition at extravascular sites of inflammation.

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