Molecular Mechanism of Tissue Factor-mediated Acceleration of Factor VIIa Activity*

(Received for publication, June 7, 1996, and in revised form, August 5, 1996)

Shouichi Higashi‡§, Naomi Matsumoto‡, and Sadaaki Iwanaga‡¶

From the ‡Department of Biology, Faculty of Science, and the ¶Department of Molecular Biology, Graduate School of Medical Science, Kyushu University 33, Fukuoka 812-81, Japan

The mechanism of the acceleration of the catalytic activity of factor VIIa (VIIa) in the presence of tissue factor (TF) was investigated. To explore the VIIa’s site(s) that correlates with TF-mediated acceleration, zymogen VII, VIIa, and active site-modified VIIa were prepared, and dissociation constants (Kd) for their bindings to TF or soluble TF in solution were determined. We found that conversion of zymogen VII to VIIa led to an increase in affinity (ΔΔG = 4.3–4.4 kJ/mol) for TFs. Dansyl-Gly-Arg-chloromethyl ketone (DNS-EGRck) treatment of VIIa led to a further increase in the affinity (ΔΔG = 7.3–12 kJ/mol). Neither removal of the Gla domain from VIIa nor truncation of the COOH-terminal membrane and cytoplasmic regions of TF affected the affinity enhanced after DNS-EGRck treatment of VIIa. Treatment of VIIa with (p-amidinophenyl)methanesulfonfonyl fluoride also enhanced its affinity for soluble TF, whereas treatment with 4-(2-aminoethyl)benzenesulfonyl fluoride had a slight effect on the affinity. On the other hand, DNS-EGRck and (p-amidinophenyl)methanesulfonfonyl fluoride treatments, but not diisopropyl fluorophosphate treatment, of VIIa led to protection of its amidinophenyl) (DNS-EGRck) treatment of VIIa. Treatment of VIIa with (p-amidinophenyl) methanesulfonfonyl fluoride, phenylmethylsulfonyl fluoride, or diisopropyl fluorophosphate had a slight effect on the affinity. On the other hand, DNS-EGRck and (p-amidinophenyl)methanesulfonfonyl fluoride treatments, but not diisopropyl fluorophosphate treatment, of VIIa led to protection of its amidinophenyl) (DNS-EGRck) treatment of VIIa. Protection of the α-amino group was consistent with formation of a critical salt bridge between Ile-153 and Asp-343 in the protease domain of VIIa. Therefore, TF may preferentially bind to the active conformational state of VIIa. When one assumes that free VIIa exists in equilibrium between minor active and dominant zymogen-like inactive conformational states, preferential binding of TF to the active state leads to a shift in equilibrium. We speculate that TF traps the active conformational state of VIIa and converts its zymogen-like state into an active state, thereby accelerating the VIIa activity.

Factor VIIa (VIIa)* is a plasma serine protease that is essen-

* This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Division of Cell Biology, Kihara Institute for Biological Research, Yokohama City University, Mioka-cho 614-12, Totsuka-ku, Yokohama 224, Japan. Fax: 045-820-1901.

§ The abbreviations used are: VIIa, factor VIIa; VII, factor VII; VII*, inactive derivative of VII; TF, tissue factor; Glu, γ-carboxyglutamic acid; VIIa(GD−), Glu domainless VIIa; DNS-EGRck, dansyl-L-glutamyl-L-arginine chloromethyl ketone; TBS, Tris-buffered saline; EGF, epidermal growth factor; PMSF, phenylmethylsulfonyl fluoride; APMSF, (p-amidinophenyl)methanesulfonyl fluoride; ABSSF, 4-(2-amidinophenyl)benzenesulfonyl fluoride hydrochloride (ABSF) from Wako Pure Chemical Industries (Osaka); diisopropyl fluorophosphate (DFP) from Katayama Chemical (Osaka); phenylmethylsulfonyl fluoride (PMSF) from Sigma, potassium cyanate from Kanto Chemical Co., Inc. (Tokyo).

EXPERIMENTAL PROCEDURES

Materials—The sources of materials used were as follows: dansyl-L-glutamyl-L-arginine chloromethyl ketone dihydrochloride (DNS-EGRck) from Calbiochem, S-2288 (H-D-Ile-Pro-Arg-p-nitroanilide dihydrochloride) from Chromogenix (Stockholm), (p-amidinophenyl)methanesulfonyl fluoride hydrochloride (APMSF) and 4-(2-amidinophenyl)benzenesulfonyl fluoride hydrochloride (ABSF) from Wako Pure Chemical Industries (Osaka); diisopropyl fluorophosphate (DFP) from Katayama Chemical (Osaka); phenylmethylsulfonyl fluoride (PMSF) from Sigma, potassium cyanate from Kanto Chemical Co., Inc. (Tokyo).
α-Chymotrypsin was purchased from Worthington. The ammonium sulfate fraction (20–60%) saturation of the urinary citrate derived from bovine plasma was a gift from Moehida Pharmaceutical Co. Ltd. (Tokyo). All other chemicals were of analytical grade or the highest quality commercially available.

**Proteins**—Bovine VII was highly purified, as described (19). VIIa and Gla domainless VIIa (VIIa(GD−)) were prepared from the bovine factor VII, as described (7). Bovine TF was purified from acetone powder of the placenta, using a modification of the method of Broze et al. (20) and was kept in the presence of 0.1% Triton X-100. The DNA encoding the extracellular domain of TF (amino acid residues 1–215, named recombinant bovine soluble TF, rsBTF) was constructed from the cDNA of bovine TF previously isolated (21). The protein was expressed in yeast and purified using the same procedures as used for human soluble TF, also expressed in yeast (22). The purified proteins gave single bands with a molecular mass of 37,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein concentrations of rsBTF, VII, and derivatives of VII were determined by amino acid analysis. The concentration of TF was determined by the dye binding method with bovine serum albumin as the standard (23), and the molecular mass used was 43,000 (24).

**Treatments of VIIa and VIIa(GD−) with DNS-EGRck**—As in previous work (7, 12), 50 μl of 5 mM DNS-EGRck dissolved in distilled water was added to 450 μl of each of the protein solutions, which contained 1.6 nmol of VIIa or 1.3 nmol of VIIa(GD−) in 50 mM Tris- HCl, pH 8.0, containing 0.1 M NaCl and 0.01% NaN3 (TBS), and each mixture was incubated at 25°C. After 4 h, excess DNS-EGRck was removed by extensive dialysis against TBS.

**Treatments of VIIa with APMSF, ABSSF, PMSF, or DFP**—Fifty microliters each of 50 mM APMSF dissolved in distilled water, 40 mM PMSF, and 20 mM DFP dissolved in methanol were added to 450 μl of the protein solution, which contained 1.6 nmol of VIIa in TBS, and the mixture was incubated at 25°C. After 4 h, excess reagents were removed by extensive dialysis against TBS. In the case of APMSF treatment of VIIa, 10 μl of 100 mM APMSF newly dissolved in distilled water was added five times every 30 min to 450 μl of the VIIa solution at 25°C. After the final addition of APMSF, incubation was continued for a further 2 h. The sample was then dialyzed against TBS.

**Determination of Dissociation Constants for Binding of VIIa or Gla Domainless VIIa to TF or rsBTF**—Various concentrations of rsBTF or TF were incubated with a fixed concentration of VIIa (2.0 or 0.2 nmol) or 2.8 nmol VIIa(GD−) in 230 μl of TBS containing 5 mM CaCl2 and 0.1% bovine serum albumin at 37°C for 15 min, and then 20 μl of S-2288 (10 mM) was added. In the presence of TF, 0.02% Triton X-100 was also added to the reaction mixture. The rates of change in absorbance at 405 nm were measured to determine the velocity of hydrolysis of S-2288. The rate of hydrolysis of S-2288 in the absence of TF was also measured, as described above, and subtracted from the total rate of the substrate hydrolysis. The data were subjected to Woolf-Augustinsson-Hofstee plot analysis (25) to determine the apparent dissociation constants ($K_d$) . In this analysis, (TF) versus (TF) [TF] were plotted; where $v$ is the velocity of hydrolysis of S-2288 by VIIa or VIIa(GD−) complexed with TF or rsBTF, and [TF] is concentration of free TF. The values of [TF] were obtained using the equation,

$$[\text{TF}] = [\text{TF}] - V_{satur}/V_{saturation}$$  

where $[\text{TF}]$ represents the overall concentration of TF. Equation 3 can be derived from Equation 2,

$$p = [\text{VII}^*]/[\text{TF}] = [\text{VII}^*]/[\text{VII}^*]/[\text{TF}] + [\text{TF}] + [\text{VII}^*]/[\text{TF}]$$  

where $K_d$ is a dissociation constant for binding of VIIa to TF, and $K_d^*$ is that for binding of VII* to TF. In the condition of $[\text{VII}^*] \gg K_d^*$ and $[\text{VIIa}] = [\text{VII}^*]$, Equation 4 can be derived from Equation 3.

$$K_d^* = K_d(1-p)/p$$  

On the other hand, one can obtain the $p$ values from the rate of hydrolysis of S-2288 by VIIa complexed with TF, using Equation 5,

$$p = V_{\text{saturation}}/V_{satur}$$

where $V_{\text{saturation}}$ is an observed velocity of hydrolysis of S-2288 by VIIa complexed with TF in the presence of VII* and $V_{\text{satur}}$ is velocity of that in the presence of a saturating concentration of VIIa. As shown in Equations 3 and 4, the $p$ value depends on the concentration of VIIa/ VII* mixture but is constant at high concentrations of the mixture. The $p$ values versus concentrations of VIIa/VII mixture were plotted to obtain the $p$ value where the constant was reached (Fig. 2). The $K_d^*$ values were calculated when the $p$ values reached the constant, using Equation 4.

**Carbamoylation of α-Amino Group of Ile-153 in Active Site-modified Derivatives of VII**—Fifty microliters of 1.0 μM KNCN were added to 200 μl of protein solutions, each of which contained 370 pmol each of VIIa, DNS-EGRck-treated VIIa, APMSF-treated VIIa, or DFP-treated VIIa in TBS containing 5 mM CaCl2. The mixture was incubated at 30°C for 0, 30, 60, 120, and 240 min. After incubation, 50 μl of each sample taken from the reaction mixture was mixed with 20 μl of 1.0 μM hydroxylamine hydrochloride, pH 8.0, and incubated for 1 h at 25°C. Each of the samples was dialyzed against TBS at 4°C and incubated with 0.4 pmol of α-chymotrypsin at 25°C for 1 h. After incubation, the reaction was terminated by adding 70 μl of 20% trichloroacetic acid, and the resulting precipitate was collected by centrifugation. The precipitate was then washed with 50 mM sodium bicarbonate, pH 8.0, and subjected to gas-phase sequencer. Phenylthiohydantoin (PTH) derivatives were detected using an Applied Biosystems 120A PTH analyzer with an on-line system, as described previously (26), and the peak areas of PTH-Ile and PTH-Val were determined simultaneously in the first cycle of the analysis.

**RESULTS**

**Determination of Dissociation Constants for Binding of Inactive Derivatives of VII to TF or rsBTF**—Complex formation between VIIa or VIIa(GD−) and TF or rsBTF was determined by measuring the rate of S-2288 hydrolysis by each complex. The data were examined using Woolf-Augustinsson-Hofstee plot analysis. As shown in Fig. 1, all four linear regression curves showed much the same slope, indicating no significant difference in amidolytic activities among VIIa-TF, VIIa-rsBTF, VIIa(GD−)-TF, and VIIa(GD−)-rsBTF complexes. The four curves intercepted the x axis at different positions, indicating that $K_d$ values for the four interactions varied. The $K_d$ values are summarized in Table I.

**Determination of Dissociation Constants for Binding of Active Derivatives of VII to TF or rsBTF**—Affinities ofzymogen VII, DNS-EGRck-treated VIIa (DNS-EGRck VIIa), and DNS-EGRck VIIa(GD−) for TF or rsBTF were estimated, using a TF-partitioning assay, as described under “Experimental Procedures.” The $K_d$ values for binding of active derivatives of VII to TF or rsBTF were calculated from $p$ values that reached the constant (Fig. 2), using the $K_d$ value for binding of VIIa to TF (0.24 nm) or that for binding of VIIa to rsBTF (14 nm), by Equation 4. The obtained $K_d$ values are also summarized in Table I.

**Effect of Gla Domain from Derivatives of VIIa on Their Affinities for TF or rsBTF**—The $K_d$ values in Table I are used to calculate differences in free energy for binding of TF or rsBTF to various derivatives of VIIa before and after deletion of
the Gla domain. As shown in Table II, deletion of the Gla domain from the derivatives of VIIa resulted in great decreases in affinities for TF ($\Delta \Delta G = 11–15$ kJ/mol), whereas the deletion resulted in only modest decreases in affinities for rsBTF ($\Delta \Delta G = 4.4–4.9$ kJ/mol).

Effect of Truncation of Membrane and Cytoplasmic Regions from TF on Affinity for Derivatives of VIIa—Affinities of TF and rsBTF for derivatives of VIIa were then compared. As shown in Table III, truncation of membrane and cytoplasmic regions led to a decrease in affinity for VII, VIIa, or DNS-EGRck-treated VIIa ($\Delta \Delta G = 5.6–10$ kJ/mol). In contrast, truncation did not affect the affinity for Gla domainless derivatives of VIIa; thus, truncation of the COOH-terminal region of TF apparently results in loss of interaction with Gla domain-dependent site in VIIa.

Effect of Activation of Zymogen VII on Affinity for TF or rsBTF—As shown in Table IV, activation of zymogen VII resulted in an increase in the affinity for TF ($\Delta \Delta G = 4.4$ kJ/mol), a similar increase in the affinity for rsBTF ($\Delta \Delta G = 4.3$ kJ/mol) was observed, which means that the enhanced affinities are probably not affected by truncation of the COOH-terminal region of TF.

Effect of DNS-EGRck Treatment of VIIa or VIIa(GD–) on Affinities for TF or rsBTF—After treatment of VIIa with DNS-EGRck, the binding affinity for TF or rsBTF was greatly enhanced ($\Delta \Delta G = 7.3–12$ kJ/mol; Table V). DNS-EGRck treatment of VIIa(GD–) also resulted in a great increase in affinity for TF (or rsBTF ($\Delta \Delta G = 12–13$ kJ/mol), suggesting that neither deletion of the Gla domain from VIIa nor truncation of the COOH-terminal region of TF affects the affinity enhanced after treatment with DNS-EGRck.

Effect of Treatment of VIIa with APMSF, ABSF, PMSF, or DFP on the Affinity of VIIa for rsBTF—The results shown in Table V suggest that incorporation of the transition state analog of substrate as DNS-EGRck into VIIa induces a conformational change in the TF-binding site in VIIa. To examine the effects of other transition state analogs of substrate on the affinity of VIIa for rsBTF, VIIa was treated with APMSF, ABSF, PMSF, and DFP, and then affinities of the inactivated derivatives of VIIa for rsBTF were estimated, using the TF-partitioning assay. As shown in Table VI, DNS-EGRck-treatment proved to be the most effective enhancer of the affinity of VIIa for rsBTF. Treatment of VIIa with APMSF also greatly enhanced the affinity, and the $K_d$ value for binding of VIIa to rsBTF was reduced to $\frac{1}{50}$ after the treatment. In contrast, treatment of VIIa with ABSF, PMSF, or DFP only slightly affected the affinity (Table VI).

Effect of DNS-EGRck Treatment of VIIa on Carbamylation Rate of $\alpha$-Amino Group of Ile-153 in VIIa Heavy Chain—Since the affinity of TF for DNS-EGRck VIIa or APMSF VIIa was much higher than that for native VIIa, the conformation of the active site-modified derivatives of VIIa may be similar to that of VIIa complexed with TF. To examine this possibility, carbamylation rates of the $\alpha$-amino group of Ile-153 in various derivatives of VIIa were compared. After incubation with

| TABLE I  | Apparent dissociation constants for various VIIa·TF complexes |
|----------|-------------------------------------------------------------|
|          | VIa | VIIa(GD–) | VII | DNS-EGRck VIIa | DNS-EGRck VIIa(GD–) |
| TF       | 0.24$^a$ | 83$^b$ | 1.3$^b$ | 0.014$^a$ | 0.87$^a$ |
| rsBTF    | 14$^a$ | 92$^a$ | 74$^b$ | 0.13$^b$ | 0.73$^b$ |

$^a$ The values were obtained from results in Fig. 1.
$^b$ The values were calculated from the $p$ values obtained in Fig. 2 and the $K_d$ value for VIIa·TF complex or that for VIIa·rsBTF complex, using Equation 4.
**Table II**

| $K_d^a$ | Before deletion | After deletion | Difference | $\Delta G^b$ | nM | -fold | $kJ \cdot mol^{-1}$ |
|---|---|---|---|---|---|---|---|
| Interaction with TF | | | | | | | |
| VIIa | 0.24 | 83 | 350 | −15 | | | |
| DNS-EGRck VIIa | 0.014 | 0.87 | 62 | −11 | | | |
| Interaction with rsBTF | | | | | | | |
| VIIa | 14 | 92 | 6.6 | −4.9 | | | |
| DNS-EGRck VIIa | 0.13 | 0.72 | 5.5 | −4.4 | | | |

$^a$ The values are the same as those in Table I.

$^b$ The values were calculated from the relationship $\Delta G = -RT \cdot \ln(K_{dp}/K_{da})$, where $K_{dp}$ and $K_{da}$ are the $K_d$ values for binding of TF to several VIIa before and after deletion of the Gla domain, respectively. $R = 8.314 \cdot J \cdot mol^{-1} \cdot K^{-1}$ and $T = 310 \ K$.

**Table III**

| $K_d^a$ | Before deletion | After deletion | Difference | $\Delta G^b$ | nM | -fold | $kJ \cdot mol^{-1}$ |
|---|---|---|---|---|---|---|---|
| VII | 1.3 | 74 | 57 | −10 | | | |
| VIIa | 0.24 | 14 | 58 | 10 | | | |
| DNS-EGRck VIIa | 0.014 | 0.13 | 9.2 | −5.6 | | | |
| VIIa(GD−) | 83 | 92 | 1.1 | −0.25 | | | |
| DNS-EGRck VIIa(GD−) | 0.87 | 0.72 | 0.83 | 0.48 | | | |

$^a$ The values are the same as those in Table I.

$^b$ The values were calculated from the relationship $\Delta G = -RT \cdot \ln(K_{dp}/K_{da})$, where $K_{dp}$ and $K_{da}$ are the $K_d$ values for binding of TF to various VIIa before and after deletion of the membrane and cytoplasmic regions of TF, respectively. $R = 8.314 \cdot J \cdot mol^{-1} \cdot K^{-1}$ and $T = 310 \ K$.

**Table IV**

| $K_d^a$ | Zymogen VII | VIIa | Difference | $\Delta G^b$ | nM | -fold | $kJ \cdot mol^{-1}$ |
|---|---|---|---|---|---|---|---|
| TF | 1.3 | 0.24 | 0.18 | 4.4 | | | |
| rsBTF | 74 | 14 | 0.19 | 4.3 | | | |

$^a$ The values are the same as those in Table I.

$^b$ The values were calculated from the relationship $\Delta G = -RT \cdot \ln(K_{dp}/K_{da})$, where $K_{dp}$ and $K_{da}$ are the $K_d$ values for binding of TF to VII before and after activation, respectively. $R = 8.314 \cdot J \cdot mol^{-1} \cdot K^{-1}$ and $T = 310 \ K$.

**Table V**

| $K_d^a$ | Before treatment | After treatment | Difference | $\Delta G^b$ | nM | -fold | $kJ \cdot mol^{-1}$ |
|---|---|---|---|---|---|---|---|
| Interaction with TF | | | | | | | |
| VIIa | 0.24 | 0.014 | 5.8 $\times$ 10$^{-2}$ | 7.3 | | | |
| VIIa(GD−) | 83 | 0.87 | 1.0 $\times$ 10$^{-2}$ | 12 | | | |
| Interaction with rsBTF | | | | | | | |
| VIIa | 14 | 0.13 | 9.3 $\times$ 10$^{-3}$ | 12 | | | |
| VIIa(GD−) | 92 | 0.72 | 7.8 $\times$ 10$^{-3}$ | 13 | | | |

$^a$ The values are the same as those in Table I.

$^b$ The values were calculated from the relationship $\Delta G = -RT \cdot \ln(K_{dp}/K_{da})$, where $R$ and $T$ are the $K_d$ values for binding of TF to various VIIa before and after treatment with DNS-EGRck, respectively. $R = 8.314 \cdot J \cdot mol^{-1} \cdot K^{-1}$ and $T = 310 \ K$.

**Table VI**

| Analogs | Structures | $K_d^a$/K$^a$ |
|---|---|---|
| DNS-EGRck | | 110 |
| APMSF | | 50 |
| ABSF | | 4.0 |
| PMSF | | 0.96 |
| DFP | | 3.5 |

$^a$ The values were calculated from $p$ values obtained in the TF-partitioning assay. See “Experimental Procedures.”

**Discussion**

We determined apparent $K_d$ values for binding of various derivatives of VIIa to TF or rsBTF and examined the interaction site(s) involved in the TF-mediated acceleration of VIIa activity. VIIa and VIIa(GD−) bound to TF or rsBTF with various $K_d$ values, and the resulting four complexes showed almost the same amidolytic activity toward the synthetic substrate S-2288 (Fig. 1). Hence, neither deletion of the Gla domain from VIIa nor truncation of the COOH-terminal membrane and cytoplasmic regions from TF affects the interaction(s) involved in the acceleration of VIIa activity. These data are consistent with documented studies (8, 22, 27). As deletion of the Gla domain from VIIa led to a great decrease in affinity for TF, the result was a modest decrease in the affinity for rsBTF (Table II). Therefore, the Gla domain-dependent site(s) in VIIa may be partially involved in the interaction with the COOH-terminal region of TF. Truncation of the COOH-terminal region of TF led to a decrease in affinity for VIIa but did not affect the affinity for Gla domainless derivatives of VIIa (Table III); hence, the COOH-terminal region of TF is involved in interaction with the Gla domain-dependent site(s) in VIIa. On the
other hand, the activation of zymogen VII leads to an increase in affinity for TF or rsBTF. DNS-EGRck treatment of VIIa further enhances the affinity for TF or rsBTF, and this enhanced affinity is not affected by removal of the Gla domain (Table V). The interaction gained after activation of zymogen VII or that gained after DNS-EGRck treatment of VIIa seems to be independent of interactions that require the Gla domain of VIIa or the COOH-terminal region of TF. The relationship among effects of various modifications on the free energy of interaction between VIIa and TF is illustrated in Fig. 4.

We reported earlier that the α-amino group of Ile-153 in VIIa is important for the Gla domain-independent interaction with TF. Moreover, the TF-induced conformational change of VIIa results in protection from carbamylation of the α-amino group of Ile-153 (12). If the observed effect of the DNS-EGRck treatment is the result of a conformational change of VIIa, similar to that induced by TF, reactivity of the α-amino group of Ile-153 with cyanate ion would change after the treatment. The results in Fig. 3 show that the α-amino group is indeed protected from carbamylation. Only derivatives of VIIa that gained a high affinity for TF after modification of the active site have a protected α-amino group of Ile-153. The data are consistent with the view that TF preferentially binds to an active conformational state of VIIa which has a tight salt bridge between Ile-153 and Asp-343.

Trypsin-like serine proteases apparently have active and zymogen-like inactive conformational states, and both states are in equilibrium (28). A salt bridge formed between the α-amino group Ile-16 and the β-carboxyl group of Asp-194 in chymotrypsin stabilizes the active state; thus, the equilibrium shifts into an active state with conversion of chymotrypsinogen to chymotrypsin (28, 29). Considering the analogy of the activation mechanism among trypsin-like serine proteases, we present in Fig. 5 a model for TF-mediated acceleration of VIIa activity. Equations pertaining to the cyclic equilibria of Fig. 5 are given below, and VIIaI, VIIaA, Kf, Kb, KdI, and KdA are defined in the figure legend.

\[
K_f = \frac{[\text{VIIaA}][\text{VIIaI}]}{[\text{TF}][\text{VIIaI}][\text{TF}]} \quad \text{(Eq. 6)}
\]

\[
K_d = \frac{[\text{VIIaA}][\text{TF}][\text{VIIaI}][\text{TF}]}{[\text{VIIaA}][\text{TF}][\text{VIIaI}][\text{TF}]} \quad \text{(Eq. 7)}
\]

\[
K_{dI} = \frac{[\text{VIIaI}][\text{TF}][\text{VIIaA}][\text{TF}]}{[\text{VIIaI}][\text{TF}][\text{VIIaA}][\text{TF}]} \quad \text{(Eq. 8)}
\]

\[
K_{dA} = \frac{[\text{VIIaA}][\text{TF}][\text{VIIaI}][\text{TF}]}{[\text{VIIaA}][\text{TF}][\text{VIIaI}][\text{TF}]} \quad \text{(Eq. 9)}
\]

Using Equations 6–10, the observed \( K_d \) value (\( K_{d\text{obs}} \)) for binding of VIIa to TF can be represented as in Equation 11.

\[
K_{d\text{obs}} = \frac{[\text{VIIaI}][\text{TF}][\text{VIIaA}][\text{TF}]}{[\text{TF}][\text{VIIaI}][\text{TF}][\text{VIIaA}][\text{TF}]} = \frac{(K_{dI} \cdot K_f + K_{dA} \cdot K_f)}{(K_f + 1)} \quad \text{(Eq. 11)}
\]

When one considers the interaction between VIIa and rsBTF (\( K_d = 14 \text{ nM} \)), using the model shown in Fig. 5, one can assume that \( K_{d\text{obs}} \) is close to the \( K_d \) value for binding of zymogen VII to rsBTF (74 nM). One can also assume that \( K_{d\text{obs}} \) is close to the \( K_d \)

![Fig. 3. Effects of various active site-modifications of VIIa on the rate of carbamylation of the NH3-terminal α-amino group of Ile-153. VIIa (3.5 μM, ●), DNS-EGRck VIIa (3.5 μM, ○), DFP VIIa (3.5 μM, △), or APMSF VIIa (3.5 μM, ▽) was incubated with 0.2 mM KNCO in TBS containing 5 mM CaCl2 at 30 °C, for the indicated times. After incubation, each sample was treated with α-chymotrypsin to remove the Gla domain, and the NH3-terminal Val and Ile of the two-chain Gla domainless derivatives of VIIa were analyzed simultaneously, using a gas phase sequencer as described under “Experimental Procedures.” A ratio of PTH-Ile to PTH-Val obtained from analysis of each of the derivatives of VIIa before incubation with KNCO is assumed to be 100%, and the ratios obtained from analyses of the carbamylated derivatives of VIIa versus time are plotted.

![Fig. 4. Relationship among effects of various modifications on the interaction between VIIa and TF. Relationship among effects of various modifications, estimated as differences in free energy for binding of TF to VIIa before and after the modifications (Tables II–V) is schematically illustrated. G-site represents Gla domain-dependent interaction site, and A-site represents the active form-dependent interaction site.]

![Fig. 5. Scheme for the equilibrium between active and inactive conformational states of VIIa in the presence of TF. VIIaI and VIIaA, respectively, represent a zymogen-like inactive state and active state of VIIa. Kf and Kb represent a constant for the equilibrium between the two conformational states of VIIa in the absence of TF and that in the presence of a saturating concentration of TF, respectively. KdI and KdA, respectively, represent dissociation constants for binding of TF to VIIaI and that of TF to VIIaA.]

G-site (~15 kJ/mol)  A-site (~16 kJ/mol)
value for the binding of DNS-EGRck VIIa to rsBTF (0.13 nM), since DNS-EGRck VIIa is speculated to be in an active conformational state. If the values of $K_{dA} = 0.13$ nM, $K_{dI} = 74$ nM, and $K_{dobs} = 14$ nM are applied to Equations 10 and 11, the values for $K_I$ and $K_D$ are calculated to be 0.0076 and 4.3, respectively, indicating that the fraction of an active conformational state is 0.75% in free VIIa and that in VIIa-rsBTF complex it is 81%. Therefore, the activity of VIIa can be accelerated 110-fold in the presence of saturating rsBTF. This assumption is in good agreement with reports in which hydrolysis of peptidyl amide or ester substrate catalyzed by VIIa is accelerated 40–150-fold in the presence of TF (5–7, 22).

DNS-EGRck and APMSF, respectively, contain guanidino and amidino groups, which are designed to interact with Asp-189 (chymotrypsin numbering) in trypsin-like serine proteases. Some specific interaction between the subsite of VIIa and the transition state analogs of its specific substrate may provide the energy required for the conformational change in VIIa. Bode et al. (30) reported that binding of a pancreatic trypsin inhibitor to trypsinogen induces an active conformational state in zymogen and leads to uptake of Ile-Val dipeptide into zymogen. This seems analogous to the conformational change of VIIa induced by transition state analogs, since the inhibitor binding coupled with the salt bridge formation led to induction of an active conformational state in zymogen.

Trypsinogen, but not trypsin, has a flexible segment named the activation domain (31). Therefore, the putative activation domain in VIIa corresponding to that in trypsinogen/trypsin is one candidate for the effecting site of TF. Waxman et al. (32) detected a segmental motion in human VIIa; using a fluorescence anisotrophy decay method, they found that the segmental motion is lost after a complex formation with TF. The putative activation domain of VIIa may be responsible for the segmental motion. During preparation of this manuscript, the crystal structure of human soluble TF-VIIa complex was reported (33). According to their data, TF does not have direct contacts with a region corresponding to the activation domain found in trypsinogen/trypsin. However, a neighboring loop corresponding to residues Cys-310 to Cys-329 of human VIIa is in contact with TF. This loop is longer than those of related serine proteases and may form an extended flexible activation domain in VIIa.

Whether the putative activation domain corresponds to the region of conformational change in VIIa will need to be determined.

Acknowledgments—We thank Mochida Pharmaceutical Co., Ltd. for kindly providing the bovine prothrombin concentrate, C. Yano for amino acid analyses, M. Ohara for helpful comments, and S. Matsumura for expert secretarial assistance.

REFERENCES

1. Silverberg, S. A., Nemerson, Y., and Zur, M. (1977) J. Biol. Chem. 252, 8481–8488
2. Nemerson, Y. (1988) Blood 71, 1–8
3. Edgington, T. S., Mackman, N., Brand, K., and Ruf, W. (1991) Thromb. Haemostasis 66, 67–79
4. Pedersen, A. H., Nordfand, O., Norris, F., Wiberg, F. C., Christensen, P. M., Moeller, K. B., Meidahl-Pedersen, J., Beck, T. C., Norris, K., Hedner, U., and Kisel, W. (1990) J. Biol. Chem. 265, 16786–16793
5. NeuenSchwanD, M., Bänzam, D. E., and Morrissey, J. H. (1993) Thromb. Haemostasis 70, 970–977
6. Lawson, J. H., Butenas, S., and Mann, K. G. (1992) J. Biol. Chem. 267, 4834–4843
7. Higashi, S., Nishimura, H., Fujii, S., Takada, K., and Iwanaga, S. (1992) J. Biol. Chem. 267, 17990–17996
8. Ruf, W., Kalnik, M. W., Lund-Hansen, T., and Edgington, T. S. (1991) J. Biol. Chem. 266, 15719–15725
9. Sakai, T., Lund-Hansen, T., Thim, L., and Kisel, W. (1990) J. Biol. Chem. 265, 1890–1894
10. Toomey, J. R., Smith, K. J., and Stafford, D. W. (1991) J. Biol. Chem. 266, 19198–19202
11. O’Brien, D. P., Kemball-Cook, G., Hutchinson, A. M., Martin, D. M. A., John-}
12. Higashi, S., Nishimura, H., Fujii, S., Takada, K., and Iwanaga, S. (1992) J. Biol. Chem. 267, 18881–18886
13. Kazama, Y., Pastuszyn, A., Wildgosse, P., Hamamoto, T., and Kisel, W. (1993) J. Biol. Chem. 268, 16231–16240
14. Wildgosse, P., Kazim, A., and Kisel, W. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 7290–7294
15. Wildgosse, P., Foster, D., Schiedt, J., Wiberg, F. C., Birktoft, J. J., and Petersen, L. C. (1993) Biochemistry 32, 114–119
16. O’Brien, D. P., Gale, K. M., Anderson, J. S., McVey, J. H., Miller, G. J., Meade, T. W., and Tuddenham, E. G. D. (1991) Blood 78, 132–140
17. MatsuShita, T., Kojima, T., Emi, N., Takahashi, I., and Saile, H. J. (1994) J. Biol. Chem. 269, 755–763
18. Kumar, A., Blumenthal, D. K., and Fair, D. S. (1991) J. Biol. Chem. 266, 915–921
19. Higashi, S., Kawabata, S., Nishimura, H., Funasaki, H., Ohyama, S., Miyamoto, S., Funatsu, A., and Iwanaga, S. (1990) J. Biol. Chem. 265, 19198–19202
20. Broze, G. J., Jr., Leykam, J. E., Schwartz, B. D., and Miletich, J. P. (1985) J. Biol. Chem. 260, 10917–10920
21. Takayenoki, Y., Miyama, T., Miyamoto, S., Takao, T., and Shimonishi, Y. (1988) J. Biol. Chem. 263, 14868–14877
22. Lu, D., Schaffner, W., and Weissmann, C. (1973) Anal. Biochem. 56, 502–514
23. Bach, R., Nemerson, Y., and Konigsberg, W. (1981) J. Biol. Chem. 256, 8324–8331
24. Segel, I. H. (1975) Enzyme Kinetics, pp. 320–329, Wiley-Interscience, New York
25. Takaya, H., Kawabata, S., Nakagawa, K., Yamamichi, Y., Miyata, T., Iwanaga, S., Takao, T., and Shimomizu, Y. (1988) J. Biol. Chem. 263, 14868–14877
26. Ruf, W., Behemtulla, A., and Edgington, T. S. (1991) J. Biol. Chem. 266, 2158–2166
27. Oppenheimer, H., Labouesse, B., and Hess, G. (1966) J. Biol. Chem. 241, 2720–2730
28. Freer, S. T., Kraut, J., Robertus, J. D., Wright, H. T., and Xuong, N. H. (1970) Biochemistry 9, 1997–2009
29. Bode, W., Schwager, P., and Huber, R. (1976) J. Mol. Biol. 118, 99–112
30. Bode, W., Fehlhammer, H., and Huber, R. (1976) J. Mol. Biol. 106, 325–335
31. Waxman, E., Laws, W. R., Laue, T. M., Nemerson, Y., and Ross, J. B. A. (1993) Biochemistry 32, 3005–3012
32. Banner, D. W., D’Arcy, A., Chen, C., Winkler, F. K., Guha, A., Konigsberg, W. H., Nemerson, Y., and Kirchofer, D. (1996) Nature 380, 41–46

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
Molecular Mechanism of Tissue Factor-mediated Acceleration of Factor VIIa Activity
Shouichi Higashi, Naomi Matsumoto and Sadaaki Iwanaga

J. Biol. Chem. 1996, 271:26569-26574.
doi: 10.1074/jbc.271.43.26569

Access the most updated version of this article at http://www.jbc.org/content/271/43/26569

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 32 references, 20 of which can be accessed free at http://www.jbc.org/content/271/43/26569.full.html#ref-list-1