Conformation of Factor VIIa Stabilized by a Labile Disulfide Bond (Cys-310–Cys-329) in the Protease Domain Is Essential for Interaction with Tissue Factor*

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Unlike other trypsin-type serine proteases, zymogen-to-enzyme transition of conformation of factor VII apparently requires not only conversion of the zymogen to active form factor VIIa (VIIa) but also interaction of VIIa with tissue factor (TF). To determine the region of interaction that correlates with maturation of the VIIa active site, we modified intramolecular disulfide bonds in VIIa and examined the interaction of the modified VIIa with soluble TF (sTF). We found that partial reduction and S-carboxamidomethylation of disulfide bonds in VIIa led to losses of amidolytic activity and the binding ability to stTF. To determine the sites of modification that associate with the loss of functions, partially S-carboxamidomethylated VIIa was separated on a column of immobilized stTF. Each of the stTF-bound and stTF-unbound fractions and native VIIa was then digested by trypsin, and the digest was analyzed by reversed-phase high performance liquid chromatography. We found that reduction and S-carboxamidomethylation of a disulfide bond between Cys-310 and Cys-329 in the protease domain of VIIa led to loss of the binding ability with sTF, and the modification of a disulfide bond between Cys-340 and Cys-368 of VIIa led to loss of the amidolytic activity. In the three-dimensional structures of trypsinogen and trypsin, the disulfide bonds corresponding to Cys-340–Cys-368 and Cys-310–Cys-329 of VIIa are, respectively, in and adjacent to the activation domain, which has flexible conformation in trypsinogen but not in trypsin. Furthermore, the crystal structure of human VIIa–TF complex indicates that the region next to Cys-310–Cys-329 is in contact with sTF. We speculate that a regional flexibility, reflected by the labile nature of disulfide bonds of Cys-310–Cys-329 and Cys-340–Cys-368 in the protease domain, contributes to the inability of VIIa to attain the active conformation. Interaction of TF with this flexible region may stabilize the structure in a conformation similar to that of the active state of VIIa.

Initiation of the extrinsic blood coagulation pathway is mediated by a complex formation between plasma-derived factor VII/VIIa and cell-bound tissue factor (TF).

VII is a trypsin-type serine protease zymogen that is initially synthesized in hepatocytes and is then secreted into the blood. The complex formation between zymogen VII and TF in the presence of Ca$^{2+}$ and phospholipids facilitates conversion of the zymogen to the active form VIIa, which is catalyzed by downstream coagulation factors or VIIa–TF complex itself. The complex formation between VIIa and TF also greatly enhances the protease activity of VIIa toward its natural substrates, factors X and IX, thus triggering the coagulation cascade (1–4). The reciprocal activation between VII/VIIa and its downstream factors after exposure to TF may be important for triggering the cascade. Although physiological initial activator of zymogen VII is unknown, the resulting VIIa still has weak protease activity and is insensitive to plasma protease inhibitors (5, 6). Therefore, trace amounts of free VIIa circulating in blood (7) without forming complexes with inhibitors may provide the capacity to initiate the coagulation pathway. In vitro, formation of the active complex can be evidenced by measuring the amidolytic activity of VIIa; this activity is also enhanced in the presence of TF and Ca$^{2+}$ without phospholipids (8–12).

To examine the molecular interaction between VIIa and TF, the sites of VIIa interacting with TF have been explored. These results suggested that the γ-carboxyglutamic acid (Gla) domain (11–13), the first epidermal growth factor-like domain (14–18), and both of the two domains (16–18) are important for binding of VIIa to TF. On the other hand, we reported that VIIa has a Gla domain-independent site(s) interacting with TF, whereas zymogen VII does not have such a corresponding site(s) and that an α-amino group of NH$_2$-terminal Ile-153 of VIIa heavy chain is important for the Gla domain-independent interaction with TF (17). Furthermore, the α-amino group of Ile-153 in VIIa is protected from carbamylation after complex formation with TF; hence the TF-induced conformational change occurs in this region. When the active site of VIIa is modified with transition state analogs of a substrate such as dansyl-Glu-Gly-Arg chloromethyl ketone or p-amidinophenylmethanesulfonyl fluoride, the α-amino group of Ile-153 is protected from carbamylation (19). Furthermore, modification of the active site of VIIa with dansyl-Glu-Gly-Arg chloromethyl ketone or p-amidinophenylmethanesulfonyl fluoride leads to a great increase in its affinity for TF. All of these data support a model where the protease domain of VIIa exists in equilibrium between minor active and dominant zymogen-like inactive conformational states, and preferential binding of TF to the active state leads to a shift in equilibrium into the active state, thereby acceler-

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1 The abbreviations used are: TF, tissue factor; TBS, Tris-buffered saline; rsTF, recombinant bovine-soluble tissue factor; PMSF, phenylmethylsulfonyl fluoride; HPLC, high performance liquid chromatography.
ating VIIa activity. Although several sites containing residues 195–206 (20), Glu-220 (21), Arg-304 (22, 23) or other sites (24) located in the protease domain of human VIIa have been suggested to interact with TF, their relation to the poor activity of free VIIa and/or TF-mediated acceleration of VIIa activity has remained to be determined. We now provide evidence that labile disulfide bonds in the protease domain of VIIa stabilize the conformations essential for catalytic activity and interaction with TF.

EXPERIMENTAL PROCEDURES

Materials—The sources of materials used were as follows: S-2288 (H-φ-Ile-Pro-γ-nitroaniline dihydrochloride) from Chromogenix, Stockholm; phenylmethylsulfonyl fluoride (PMSF) from Sigma; Affi-Gel 10 from Bio-Rad; 2-mercaptoethanol and iodoacetamide from Nacalai Tesque Inc., Kyoto; Ultrasphere ODS 5U (2.0 × 150 mm) from Beckman. Bovine pancreatic trypsin treated with N-ethyl-p-phenylenechloromethyl ketone was purchased from Worthington. The ammonium sulfate fraction (40–65% saturation) of the barium citrate precipitate from bovine plasma was a gift from Mochida Pharmaceutical Co. Ltd., Tokyo. All other chemicals were of analytical grade or the highest quality commercially available.

Proteins—Bovine VIIa was highly purified, as described (25). VIIa was prepared from the bovine VII, as described (11). The DNA encoding the extracellular domain of VIIa (amino acid residues 1–213, named recombinant bovine-soluble TF, rsTF) was constructed from the cDNA of bovine TF previously isolated (26). The protein was expressed in yeast and purified using the same procedures as those used for human soluble TF, also expressed in yeast (5). The purified rsTF gave a single band when visualized by staining with iodoacetamide; the concentration in the reaction mixture was adjusted to 1.2-fold molar excess of 2-mercaptoethanol. After 2 h, the samples were dialyzed against TBS.

Assay of Amidolytic Activity of VIIa after Modification—After modification of VIIa, under various conditions the modified VIIa (20 nm) was incubated with rsTF (600 nm) in 230 µl of TBS containing 5 mM CaCl2 and 0.1% bovine serum albumin at 37 °C for 15 min; then 20 µl of S-2288 (10 mM) was added. 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 by the modified VIIa (400 nm) in the absence of rsTF was also measured, as described above. The rate of hydrolysis of S-2288 without enzyme was measured as described above and subtracted from the total rate of the substrate hydrolysis.

Treatment of Partially Reduced and S-Carboxamidomethylated VIIa with PMSF—Five microliters each of 50 mM PMSF dissolved in methanol was added to 45 µl of the protein solution, which contained 0.23 nmol of partially reduced and S-carboxamidomethylated VIIa in TBS, and the mixture was incubated at 25 °C. After 4 h, excess reagent was removed by extensive dialysis against TBS.

Inhibition Assay of Amidolytic Activity of VIIa + rsTF Complex by PMSF-treated Derivatives of VIIa—VIIa (14 nm) and rsTF (20 nm) were incubated with various concentrations of PMSF-treated derivatives of VIIa in 230 µl of TBS containing 5 mM CaCl2 and 0.1% bovine serum albumin at 37 °C for 15 min; then 20 µl of S-2288 (10 mM) was added. 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 rsTF was also measured as described above and subtracted from the total rate of the substrate hydrolysis.

Separation of rsTF-bound and rsTF-unbound Forms of VIIa after Partial Reduction and S-Carboxamidomethylation of VIIa—VIIa (1.2 mg) was incubated with 15 mM 2-mercaptoethanol in 5 ml of TBS containing 5 mM CaCl2 at 25 °C for 60 min. After incubation, the sample was transferred to an ice-water container and further incubated with 18 mM iodoacetamide in the dark. After 2 h, the sample was dialyzed extensively against TBS containing 5 mM CaCl2 at 4 °C. The preparation was then applied to an rsTF-Affi-Gel 10 column (2.8 mg of rsTF was coupled to 4 ml of Affi-Gel 10), and the flow-through fraction was collected. After washing the column with TBS containing 5 mM CaCl2, the adsorbed sample was eluted with TBS containing 2 mM guanidine hydrochloride. The samples in flow-through and eluted fractions were collected and dialyzed against TBS, respectively.

Amino Acid Analysis—Samples were hydrolyzed at 110 °C for 24, 48, and 72 h in 5.7 M HCl in evacuated tubes. The hydrolysates were analyzed on a Hitachi L-8500 automated analyzer (27).

Amino-terminal Sequence Analysis—Samples were analyzed on an Applied Biosystems 477A gas phase sequencer. Phenylthiohydantoin derivatives were detected using an Applied Biosystems 120A phenylthiohydantoin analyzer with an on-line system, as described previously (28).

RESULTS

Effect of Concentration of 2-Mercaptoethanol on the Reduction of Intramolecular Disulfide Bonds in VIIa—VIIa was treated with various concentrations of 2-mercaptoethanol, and the resulting material was S-carboxamidomethylated by treating with iodoacetamide. The S-carboxamidomethylated cysteine residues in the modified VIIa were determined as S-carboxymethylcysteine by amino acid analysis. We also measured the amidolytic activity of the modified VIIa in the presence and absence of rsTF. As shown in Fig. 1, the amidolytic activity of VIIa alone was sharply diminished with increasing concentrations of 2-mercaptoethanol. The amidolytic activity of VIIa in the presence of rsTF was similarly diminished. Fifty percent reduction of the amidolytic activity of VIIa alone was observed at 3 mM mercaptoethanol. At the 50% loss of the activity, about 1.2 mol of S-carboxymethylcysteine, corresponding to a modification of 0.6 mol of disulfide bond/mol of VIIa, was estimated, suggesting an association between modification of one or few labile disulfide bonds in VIIa and loss of the amidolytic activity.

Effect of Partial Reduction and S-Carboxamidomethylation of VIIa on the rsTF-Binding Ability—To examine the rsTF binding ability of S-carboxamidomethylated VIIa, the active site of the modified VIIa was masked by a treatment with
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Enhanced affinity for rsBTF. The rsBTF binding ability of VIIa suggests a production of molecular species that have almost the same affinity for rsBTF (19), the observed increase in IC50 value at low 2-mercaptoethanol concentrations suggests a production of molecular species that have enhanced affinity for rsBTF. The rsBTF binding ability of VIIa was abolished at 50 mM 2-mercaptoethanol (Fig. 2B). During the complete loss of the binding ability, about 5.0 mol of S-carboxymethylcysteine, corresponding to a modification of 2.5 mol of disulfide bond/mol of VIIa, was estimated (Fig. 1).

Separation of rsBTF-bound and rsBTF-unbound Fractions after Partial Modification of VIIa—As described under “Experimental Procedures,” VIIa was treated with 15 mM 2-mercaptoethanol and 18 mM iodoacetamide. This modification led to loss of >90% amidolytic activity of VIIa (Fig. 1). The partially modified VIIa was then separated on an rsBTF-Affi-Gel column. After the separation, rsBTF-bound and rsBTF-unbound fractions contained almost the same amount of protein (data not shown), suggesting that 50% of the modified VIIa before separation had essentially no affinity for rsBTF. As shown in Table I, 3.5 mol of S-carboxymethylcysteine/mol of protein was estimated in amino acid analysis of the modified VIIa before separation. Therefore, only 1.8 mol of disulfide bond in a total of 12 mol of disulfide bond/mol of VIIa was modified and accompanied by 50% loss of the rsBTF binding ability. On the other hand, 2.4 mol of S-carboxymethylcysteine/mol of protein was estimated in amino acid analysis of the rsBTF-bound fraction, whereas 4.8 mol of S-carboxymethylcysteine/mol of protein was estimated for the rsBTF-unbound fraction. The difference of S-carboxymethylcysteines between the two fractions corresponds to a modification of 1.2 disulfide bonds, suggesting that modification of a single disulfide bond led to loss of rsBTF binding ability of VIIa. In the absence of rsBTF, amidolytic activity of the rsBTF-bound and rsBTF-unbound fractions relative to native VIIa were 7.2 and 6.2%, respectively (Fig. 3A). The amidolytic activity of rsBTF-bound fraction but not rsBTF-unbound fraction was enhanced in the presence of rsBTF as expected (Fig. 3B). In the presence of rsBTF, amidolytic activity of the rsBTF-bound and rsBTF-unbound fractions relative to native VIIa were 95% and 0.045%, respectively. We further examined the rsBTF binding ability of the two fractions after treatment with PMSF (Fig. 4). As compared with the PMSF-treated VIIa (IC50 = 60 nM), the modified VIIa in rsBTF-bound fraction (IC50 = 40 nM) had an enhanced affinity for rsBTF and effectively inhibited the

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**TABLE I**

| Amino acid | Pre-column | Bound | Unbound | Deduced from sequence (Ref. 28) |
|------------|------------|-------|---------|---------------------------------|
| Residues/molecule | | | | |
| Cmc | 3.5 | 2.4 | 4.8 | |
| Asp | 30.5 | 30.1 | 31.1 | 31 |
| Thr | 14.0 | 14.1 | 13.7 | 15 |
| Ser | 19.8 | 20.3 | 20.8 | 22 |
| Glu | 49.7 | 50.1 | 50.8 | 51 |
| Pro | 26.5 | 25.5 | 25.9 | 26 |
| Gly | 49.2 | 49.0 | 50.0 | 47 |
| Ala | 31.0 | 31.0 | 31.0 | 31 |
| Val | 26.5 | 27.0 | 27.1 | 29 |
| Cys | ND* | ND | ND | 12 |
| Met | 4.1 | 4.0 | 3.9 | 4 |
| Ile | 7.8 | 7.7 | 8.0 | 8 |
| Leu | 36.4 | 36.2 | 36.1 | 37 |
| Tyr | 8.8 | 8.5 | 9.2 | 9 |
| Phe | 16.4 | 16.0 | 16.8 | 17 |
| Lys | 9.5 | 9.6 | 9.9 | 10 |
| His | 10.2 | 9.7 | 9.5 | 10 |
| Trp | ND | ND | ND | 8 |
| Arg | 29.2 | 29.5 | 28.6 | 28 |

* Calculated from average values obtained from 24, 48, and 72 h hydrolyses with 5.7 M HCl.
* S-Carboxymethylcysteine.
* Extrapolated values to 0 h.
* Taken from 72-h hydrolysis.
* ND, not determined.
amidolytic activity of VIIarsBTF complex. In contrast, the rsBTF-unbound fraction did not show a significant affinity for rsBTF. These data and Fig. 2B are consistent with the view that the modification of VIIa with 15 mM 2-mercaptoethanol leads to at least two forms of VIIa, one of which has increased affinity for rsBTF and the other of which has essentially no affinity for rsBTF.

**Determination of the Modified Disulfide Bonds Responsible for the Losses of rsBTF-binding Ability and Amidolytic Activity—**To determine location of the modified disulfide bond responsible for the loss of rsBTF binding ability, the samples in rsBTF-bound and rsBTF-unbound fractions were subjected to tryptic digestion, after which the digests were separated by reversed-phase HPLC. The observed differences between the elution profiles were only U-8 and U-12 from the rsBTF-unbound fraction and B-14 from the rsBTF-bound fraction (Fig. 5, B and C). Based on the amino acid analyses (Table II), U-8 and U-12 were assigned as the peptides corresponding to residues 305–315 and 316–337 of bovine VII (28), respectively. On the other hand, B-14 was assigned as the peptide corresponding to residues 305–337 of bovine VII. As shown in Table II, 1.1 and 1.2 mol of S-carboxymethylcysteine/mol of peptides were detected in the amino acid analyses on U-8 and U-12, respectively, whereas S-carboxymethylcysteine was not detected in the analysis on B-14. Furthermore, the LLTQDXLQQSR and QRPGBPVTDT sequences corresponding to residues 305–315 and 316–325 of bovine VII were determined, respectively, in the NH2-terminal sequence analyses on U-8 and U-12. As expected, the NH2-terminal sequence analyses on B-14 showed the LLTQDXLQQSR and QRPGBPVTDT sequences. These results indicate that B-14 is a two-chain peptide derived from the protease domain of VIIa corresponding to residues 305–315 and 316–337 linked through a disulfide bond between Cys-310 and Cys-329, whereas U-8 and U-12 are single-chain peptides corresponding to residues 305–315 and 316–337, respectively, of which cysteine residues have been S-carboxamidomethylated. The results also suggest that reduction and S-carboxamidomethylation of a disulfide bond between Cys-310 and Cys-339 in the protease domain of VIIa led to a loss of binding ability with rsBTF. On the other hand, the modified VIIa in rsBTF-bound fraction contained 1.2 mol of modified disulfide bonds/mol of protein and showed only a 7.2% relative amidolytic activity and slightly enhanced affinity for rsBTF. Therefore, modification of a single disulfide bond must associate with the loss of amidolytic activity and increase in affinity for rsBTF. To determine the modified disulfide bond, native VIIa was subjected to tryptic digestion, and the digests were also analyzed by reversed-phase HPLC (Fig. 5A). The elution profile was compared with that of rsBTF-bound fraction (Fig. 5B). The observed differences between the elution profiles were only N-18 from the native VIIa and B-19 from the rsBTF-bound fraction. The NH2-terminal sequence analysis on N-18 showed the DAXK and GTWFLTGVV sequences. Based on the amino acid analysis (Table II), N-18 was assigned as the peptide corresponding to residues 338–341 and 354–379 of bovine VII (28), indicating that N-18 is a two-chain peptide derived from the protease domain of VIIa corresponding to residues 338–341 and 354–379 linked through a disulfide bond between Cys-340 and Cys-368. In contrast, the GTWFLTGVV, but not the
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DISCUSSION

To explore the site of VIIa involved in the interaction with TF, we modified intramolecular disulfide bonds of VIIa under controlled conditions and identified disulfide bonds essential for the interaction with TF and catalytic activity. We also examined reactivity of the essential disulfide bonds with 2-mercaptoethanol by monitoring the amidolytic activity and the rsBTF binding ability of VIIa after modification. We found that the amidolytic activity of VIIa was sharply diminished during reduction and S-carboxamidomethylation of a part of the disulfide bonds in VIIa (Fig. 1), suggesting that a very labile disulfide bond is important for the amidolytic activity of VIIa. On the other hand, rsBTF binding ability of VIIa was also reduced with increasing concentrations of 2-mercaptoethanol. To determine which modified disulfide bonds are associated with loss of the functions, a partially modified VIIa was separated on an immobilized rsBTF column, and the separated samples were examined for their amidolytic activity, affinity for rsBTF, and location of their modified disulfide bonds. The result suggests that reduction and S-carboxamidomethylation of a disulfide bond between Cys-310 and Cys-329 in VIIa leads to loss of the binding ability with rsBTF. The crystal structure of human soluble TF-VIIa complex indicates that the region next to Cys-310–Cys-329 is in contact with soluble TF (29). Furthermore, alanine scanning mutagenesis analysis of human VIIa recently reported by Dickinson et al. (30) indicates that the contact region containing residues of Met-306 and Asp-309 in human VIIa is essential for the affinity for human TF. We speculate that some structural elements in the contact region of VIIa are necessary for the affinity for rsBTF, and that such structural elements are destabilized with scission of the disulfide bond between Cys-310 and Cys-329. We also found that the modification of a disulfide bond between Cys-340 and Cys-368 in the protease domain of VIIa led to loss of amidolytic activity and slight increase in affinity for rsBTF. This disulfide bond exists in the vicinity of the S1 site of VIIa and may contribute to the recognition of substrates. Indeed, Varallyay et al. (31) revealed that an elimination of the disulfide bond Cys-191–Cys-220 of trypsin corresponding to Cys-340–Cys-368 of VIIa leads to great decreases in the catalytic activity and specificity of trypsin. We previously presented a model for TF-mediated acceleration of VIIa activity where the protease domain of VIIa exists in equilibrium between minor active and dominant zymogen-like inactive conformational states. In this model, preferential binding of TF to the active state leads to a shift in equilibrium into the active state, thereby accelerating VIIa activity (19). A part of the binding energy of the interaction between VIIa and TF is presumed to be consumed to drive this conformational change of VIIa. Therefore, interaction of TF with the ground state of VIIa must contain some energetically unfavorable (repulsive) elements that are directly responsible for the TF-induced activation of VIIa. We speculate that the favorable interaction of TF with a region next to the disulfide bond Cys-310–Cys-329 provides an energy required for overcoming the unfavorable elements, thereby contributing to the conformational change of VIIa and stabilization of the interaction between TF and the protease domain of VIIa. On the other hand, the observed increase in the affinity for rsBTF after modification of the disulfide bond between Cys-340 and Cys-368 of VIIa may be explained as a perturbation of the active site of VIIa upon the scission of the disulfide bond, which effects a conformation of the ground state of VIIa thus eliminating a part of the energetically unfavorable elements. In our previous study, such a slight increase in affinity of VIIa for rsBTF has been observed when the active site of VIIa is modified with diisopropyl fluorophosphate (19). However, further

DAXX, sequence was determined in the sequence analysis on B-19, as expected. S-Carboxymethyl cysteine was detected in the amino acid analysis on B-19. However, the determined composition of amino acids was not consistent with the peptide corresponding to residues 354–379 of bovine VII (data not shown), probably because B-19 had not been well separated from other peptides in the reversed-phase HPLC (Fig. 5B). Unfortunately, an S-carboxamidomethylated peptide corresponding to residues 338–341 was missing in the elution profile of rsBTF-bound fraction. The small hydrophilic peptide may have passed through the column of reversed-phase HPLC. However, the results suggest that reduction and S-carboxamidomethylation of a disulfide bond between Cys-340 and Cys-368 in the protease domain of VIIa led to a loss of amidolytic activity and a slight increase in affinity for rsBTF.

Fig. 5. HPLC separation of tryptic peptides of VIIa or rsBTF-bound and rsBTF-unbound fractions of partially reduced and S-carboxamidomethylated VIIa. After partial reduction and S-carboxamidomethylation, the modified derivatives of VIIa were separated using an rsBTF-Affi-Gel 10 as described under “Experimental Procedures.” Each of the samples obtained and native VIIa was then digested with trypsin in an enzyme to a substrate ratio of 1:100 (w/w) at 37 °C for 24 h. The digest of native VIIa (panel A), rsBTF-bound fraction (panel B), or rsBTF-unbound fraction (panel C) was applied to an Ultrasphere ODS 5U column (2.0 × 150 mm) and eluted at a flow rate of 0.5 ml/min with a linear gradient of acetonitrile containing 0.1% trifluoroacetic acid. The column eluate was monitored at 210 nm (solid lines), and the broken line shows the percentage of acetonitrile in the elution medium. B, bound; U, unbound.
study will be required to clarify these mechanisms.

Trypsinogen, but not trypsin, has a flexible segment named
activation domain (32). Considering the analogy of structure
among trypsin-type serine proteases, the putative activation
domain in VIIa corresponding to that in trypsinogen/trypsin is
a prime candidate for the site of effects of TF. In trypsinogen/
trypsin, the disulfide bonds corresponding to Cys-340–Cys-368
and Cys-310–Cys-329 of VIIa are, respectively, in and adjacent
to the activation domain (Fig. 6). The labile nature of the
disulfide bonds of VIIa observed in the present study may well
reflect flexibility of the putative activation domain and its
flanking region of VIIa. We speculate that the high flexibility of
a region around the disulfide bond between Cys-310 and Cys-
329 contributes to the enhanced mobility of the adjacent acti-
vation domain in VIIa, thus making it unable for VIIa to gain
active conformation in the absence of TF. Waxman et al. (33),
who detected a segmental motion in human VIIa using a fluo-
rescence anisotropy decay method, found that the segmental
motion is lost after a complex formation with TF. Interaction
of TF with a flexible region around the disulfide bond may asso-
ciate with the loss of motion of the activation domain, thereby
contributing to stabilization of the active conformational state
of VIIa. Recent studies on natural mutation of human VII suggest that substitutions of Arg-304
Gln/Trp (22, 23), Phe-328
Ser (34), and Cys-310
Phe (35)
in the protease domain have an adverse effect on TF interac-
tion. All of the sites of substitution are in close proximity, and
the last substitution results in the lack of a disulfide bond
between Cys-310 and Cys-329, which also suggests the impor-
tance of this region for interaction with TF. Whether or not the
flexibility of a region around the disulfide bond between Cys-
310 and Cys-329 contributes the motion of putative activation
domain in VIIa will need to be determined.

Fig. 6. Sequence around the disulfide bond corresponding to Cys-310–Cys-329 of VIIa in several serine proteases. S–S represents
the disulfide bond corresponding to Cys-310–Cys-329 of VIIa in several serine proteases. Boxed area shows a part of the activation domain found
in trypsinogen/trypsin. Ile-153 (Ile-16), the site of the critical salt bridge formed between α-amino group of the NH₂-terminal amino acid and
aspartate side chain adjacent to the catalytic serine in active enzymes; S1 residue, the site of interaction with P1 residue of substrate; *, catalytic
serine; R₃Q₀ or W, F → S, and C → F, natural mutations found in human VII (22, 23, 34, 35). The bottom numbers represent chymotrypsin
numbering. B, bovine; H, human; PC, protein C; Th, thrombin; Tryp, trypsin; Chy, chymotrypsin.

| Amino acid | U-8 (Residues 305–315) | U-12 (Residues 316–337) | B-14 (Residues 305–315 and 316–337) | N-18 (Residues 338–341 and 354–379) |
|------------|------------------------|------------------------|----------------------------------|----------------------------------|
| Cmcᵇ       | 1.1                    | 1.2                    | 0.0                              | 0.0                              |
| Asp        | 1.1 (1)                | 2.8 (3)                | 4.5 (4)                          | 1.1 (1)                          |
| Glu        | 2.7 (3)                | 1.2 (1)                | 4.1 (4)                          | 1.2 (1)                          |
| Ser        | 0.6 (1)                | 1.6 (2)                | 2.2 (3)                          | 0.5 (6)                          |
| Gly        | 0.0 (0)                | 3.4 (4)                | 3.7 (4)                          | 5.5 (6)                          |
| His        | 0.0 (0)                | 0.0 (0)                | 0.0 (0)                          | 1.0 (1)                          |
| Arg        | 1.0 (1)                | 1.2 (1)                | 1.0 (1)                          | 4.0 (4)                          |
| Thr        | 1.1 (1)                | 0.9 (1)                | 2.0 (2)                          | 2.7 (3)                          |
| Ala        | 0.0 (0)                | 1.0 (1)                | 1.0 (1)                          | 1.2 (1)                          |
| Pro        | 0.0 (0)                | 2.2 (2)                | 2.4 (2)                          | 0.0 (0)                          |
| Tyr        | 0.0 (0)                | 1.2 (1)                | 0.9 (1)                          | 0.9 (1)                          |
| Val        | 0.0 (0)                | 1.7 (2)                | 1.8 (2)                          | 1.9 (2)                          |
| Met        | 0.0 (0)                | 0.9 (1)                | 0.7 (1)                          | 0.0 (0)                          |
| 1/2 Cysᵈ   | 0.0 (1)                | 0.0 (1)                | 1.7 (2)                          | 1.7 (2)                          |
| Ile        | 0.0 (0)                | 0.0 (0)                | 0.0 (0)                          | 1.0 (1)                          |
| Leu        | 2.9 (3)                | 0.0 (0)                | 3.4 (3)                          | 1.2 (1)                          |
| Phe        | 0.0 (0)                | 1.1 (1)                | 1.2 (1)                          | 2.2 (2)                          |
| Trp        | NDᵇ (0)               | ND (0)                 | ND (0)                           | ND (2)                           |
| Lys        | 0.0 (0)                | 0.9 (1)                | 1.1 (1)                          | 1.1 (1)                          |

² Calculated from the 20-h hydrolysate with 5.7 M HCl.
ᵇ S-Carboxymethylcysteine.
ᶜ Values in parentheses are taken from the sequence data of bovine factor VII (Ref. 28).
ᵈ Determined as cysteic acid after performic acid oxidation.
e ND, not determined.
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