THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 266, No. 2, Issue of January 15, pp. 915–921, 1991
Printed in U.S.A.

Identification of Molecular Sites on Factor VII Which Mediate Its Assembly and Function in the Extrinsic Pathway Activation Complex*

(Received for publication, July 24, 1990)

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Factor VII-VIIa, in association with tissue factor, participates in the complex which initiates blood coagulation through the extrinsic pathway. To identify functional domains on factor VII which mediate the activation of factor X, 16 synthetic peptides corresponding to 55% of the primary structure were assayed for their ability to inhibit factor VII function. Factor Xa formation was inhibited by eight of the peptides in a dose-dependent manner. Kinetic analyses indicated noncompetitive inhibition of factor X activation by seven of these peptides. Peptide-(347–361) inhibited factor Xa cleavage of a chromogenic substrate by a competitive mechanism and was excluded from further analysis in this study. Among the seven inhibitory peptides which have the ability to prevent the factor VIIa-tissue factor-mediated conversion of factor X to factor Xa, peptide-(285–305) was most inhibitory, with a K_i value of 2.4 μM. The K_i values were in the range of 42–65 μM for peptides-(44–50), -(194–214), -(208–229), and -(376–390). The least inhibitory peptides were at positions 170–178 and 330–340, with a K_i value >200 μM. Polyclonal antibodies were raised against four of these peptides; and when antisera were assayed by a solid-phase radioimmunoassay, they bound not only to their respective immunizing peptides, but also to factor VII. The Fab fragments of specific IgG preparations, affinity-purified on a factor VII-agarose column, inhibited the rate of factor X activation in a dose-dependent manner. Six of the seven inhibitory peptides represent amino acid sequences within the heavy chain of factor VII, and the remaining one corresponds to a sequence within the light chain. The corresponding regions in the x-ray crystal structure of chymotrypsin represented by the six heavy chain inhibitory peptides are found to be located in three distinct regions, one region located spatially distal to the active site and the other two regions located relatively closer to the active site and the substrate-binding pocket. The results suggest that at least three specific regions in the heavy chain and one region in the light chain of factor VII mediate its interaction with the factor X activation complex.

Coagulation is initiated by the expression of tissue factor, a nonproteolytic integral membrane receptor/cofactor of factor VII, by either extravascular cells upon injury or vascular cells on stimulation by a variety of biological agonists (1, 2). This factor VII-VIIa-tissue factor complex activates factor X to its active enzyme form (Xa), which leads to the formation of clots by the extrinsic pathway (1–3). In addition, this complex can activate factor IX to factor IXa and promote clotting by recruiting the intrinsic pathway (4).

Factor VII, a trace vitamin K-dependent serine protease zymogen, is synthesized and secreted as a single-chain molecule consisting of 406 amino acid residues (5–7). The activation of factor VII to factor VIIa involves the hydrolysis of a single peptide bond between Arg^{152} and Ile^{153}, the resulting two-chain molecule (VIIa) consisting of a light chain of 152 amino acid residues and a heavy chain of 254 amino acid residues held together by a single disulfide bond (7). The light chain of factor VIIa contains the 10 γ-carboxylglutamic acid residues and two growth factor homology domains, whereas the heavy chain contains the catalytic domain typical of serine proteases. Factor VII, unlike other zymogen forms, can react with diisopropyl fluorophosphate, a potent inhibitor of serine proteases (6, 9). In addition, bovine factor VII exhibits ~1–2% of the coagulant activity of factor VIIa (5). This has led to the suggestion that factor VII is an "active zymogen." However, recent studies (10, 11) provide evidence to show that human factor VII is a true zymogen and must be cleaved prior to the expression of proteolytic activity. The complex of factor VIIa-tissue factor in the presence of Ca^{2+} on a phospholipid membrane surface rapidly activates both factors X and IX (3, 4), whereas only a minimal amount of coagulant activity is observed in the absence of tissue factor (12). By *in vitro* experiments, a number of activated coagulation proteins including factors Xa (5), IXa (13), and XIIa (5, 13) and thrombin (9) have been shown to activate factor VII to factor VIIa.

The primary structure of factor VII bears considerable sequence similarity to the family of vitamin K-dependent coagulation proteins (8). Furthermore, the catalytic domain of factor VII, like other members of the vitamin K-dependent proteins, shows a marked sequence and predicted structural homology to the pancreatic serine proteases trypsin and chymotrypsin (14–18). However, the presence of calcium, protein cofactors, and membrane surfaces is essential for the rapid activation of coagulation serine proteases; and most of them also exhibit a high degree of specificity for a limited number of plasma protein substrates. These specific interactions of vitamin K-dependent serine proteases must relate to the extended molecular recognition sites on their surface. These sites of molecular recognition may be derived from a contiguous sequence of amino acids or by a combination of discontiguous sequences which are in close spatial proximity at the surface of the molecule as a result of folding of the proteins.

* This work was supported in part by Research Grant HL 37770 from the National Institutes of Health. 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.

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Molecular interaction requires that the regions mediating these associations must be at the solvent-accessible surface of the molecule. We have used two approaches to examine the regions on factor VII which mediate assembly and function of the extrinsic pathway activation complex. Synthetic peptides representing ~55% of the primary structure of factor VII and selected antipeptide antibodies were used to conclude that at least three specific regions on the heavy chain and one region on the light chain of factor VII mediate its interaction with the factor X activation complex.

EXPERIMENTAL PROCEDURES

Materials—Factors VII-VIIa (19), X (20), and Xa (21) were isolated as previously reported. Tissue factor was prepared from human brain as described earlier (22). *t*-Butoxycarbonyl-derivatives were obtained from Chemical Dynamics Corp. (South Plainfield, NJ) and Peninsula Laboratories, Inc. (Belmont, CA), and 9-fluorenylmethoxycarbonyl-derivatives were bought from Milligen/Biosearch (Bedford, MA), Calbiochem, or Peninsula Laboratories, Inc. Glutaraldehyde (25%), cyanogen bromide, keyhole limpet hemocyanin, and rabbit brain phospholipids (cephalin) were purchased from Sigma. Sepharose 4B and Sephadex G-25 were obtained from Pharmacia LKB Biotechnology Inc. The chromogenic substrate for factor Xa, CBS 31.39 (CH_3SO_4-D-Leu-Gly-Arg-*p*-nitroanilide-AcOH), was bought from American Byproducts Co. (Parsippany, NJ). Protein A-agarose, papain-agarose, bicinchoninic acid protein reagent, *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester, and IODO-GEN iodination reagent were obtained from Pierce Chemical Co. All other reagents were of the highest purity available.

Peptide Synthesis and Characterization—Peptides with amino acid sequences corresponding to the primary structure of factor VII, numbered according to Hagen *et al.* (8), are listed in Table I. First-generation peptides were synthesized by the Merrifield solid-phase methodology as described by Glass (23). Synthesis was performed on a Biosearch SAM II synthesizer at the 1-meq scale. All the residues were double-coupled using a 3-fold molar excess of each *t*-butoxycarbonyl-derivative to benzylhydrylamine resin. Deblocking was performed with 30% trifluoroacetic acid in dichloromethane for 30 min. Peptides were cleaved from the resin, and side chain deprotection was achieved by treatment with anhydrous hydrofluoric acid (HF) containing 20% anisole for 45 min at 0 °C. Peptides containing histidine were also treated with 2-mercaptoethanol before HF cleavage to remove the dinitrophenyl side chain protecting group. All the subsequent peptides were made manually in a RAMPs™ peptide synthesizer (Du Pont-New England Nuclear) using the 9-fluorenylmethoxycarbonyl chemistry following the manufacturer's protocols for synthesis and cleavage.

All the peptides were initially gel-filtered on a Bio-Rad P-2 column (2.5 × 83 cm) equilibrated in 1% acetic acid. Subsequent analysis and purification were performed on a Perkin-Elmer high pressure liquid chromatography system using a Vydac C_4 column (2.1 × 25 cm, 5 μm) equilibrated in 0.1% trifluoroacetic acid and developed with a linear gradient of 0–50% acetonitrile over 50 min. The composition and concentration of isolated peptides were determined by subjecting them to 24-h hydrolysis in 6 N HCl in evacuated tubes at 110 °C and by subsequent analysis on a Beckman Model 6300 amino acid analyzer.

Preparation, Characterization, and Purification of Polyclonal Antipeptide Antibodies—Antibodies were raised in rabbits against selected synthetic peptides coupled to keyhole limpet hemocyanin. One mg of *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester was dissolved in 15 μl of dimethylformamide and mixed with 5 mg of keyhole limpet hemocyanin previously dissolved in 1 ml of 20 mM phosphate, 150 mM NaCl, pH 7.4 (PBS). After 30 min of incubation at ambient temperature, the low molecular weight reactants were separated from keyhole limpet hemocyanin/*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester by gel filtration using a Sephadex G-25 column (1 × 18 cm) equilibrated in PBS. This protein was reacted with 5 mg of a cysteine-containing peptide for 3 h at room temperature. The peptide-carrier conjugates (300 μg) in 150 μl of PBS were emulsified with an equal volume of Freund's complete adjuvant and injected intradermally into four sites, one over each limb. After 2 weeks, the conjugate was emuslified in Freund's incomplete adjuvant and injected subcutaneously into four sites, one over each limb. The animals were challenged every month thereafter, and sera were collected weekly, starting 6 weeks after the initial immunization.

Sera were tested for reactivity against factor VII and free peptides using a solid-phase radioimmunoassay as described previously (24). Purification of IgG from rabbit antisera was achieved by affinity chromatography on protein A-agarose (24). The concentration of IgG was determined by the bicinchoninic acid protein assay method using bovine serum albumin as standard. Specific antibodies were purified by passing the IgG fraction of each peptide antiserum over a 10-ml column of factor VII coupled to agarose (25) at a concentration of 0.5 mg of factor VII/ml of agarose beads. The antibody bound to the column was eluted with 100 mM glycine HCl, pH 3.0; concentrated using Amicon PM-10 membranes; and dialyzed against PBS. For selected studies, monovalent Fab fragments were prepared by papain digestion of the specific antibody (24).

Rate of Factor X Activation—The effect of synthetic peptides on the rate of factor X activation was analyzed in a purified system employing a chromogenic assay (24). For inhibition experiments, peptides were diluted in 20 mM Tris-HCl, 150 mM NaCl, pH 7.4 (TBS), and preincubated with 10 μl each of factor X (12 nM) and tissue factor (0.075%) at 37 °C in a total volume of 140 μl, adjusted with TBS containing 1% bovine serum albumin and 5 mM $CaCl_2$. After 30 min, factor X activation was initiated by the addition of 10 μl of factor VII (0.2 pM). At defined time intervals, 20-μl aliquots were removed and added to a microtiter plate containing 80 μl of 50 mM Tris-HCl, 225 mM NaCl, and 10 mM EDTA, pH 8.2. Following sample collection, 50 μl of 0.6 mM chromogenic substrate (CBS 31.39) was added, and the rate of change in the absorbance at 405 nm was monitored for 10 min using a V_{max} kinetic microplate reader (Molecular Devices, Palo Alto, CA). The rate of factor Xa formation was calculated from the slope of a linear regression of the data plotted as a function of the initial rate of hydrolysis (A_{405}/min) *versus* time and expressed as A_{405}/min^2.

Coagulation Assays—The effect of synthetic peptides on factor VII coagulation activity was determined using a two-stage clotting assay as described earlier (24). Synthetic peptides were individually preincubated with 20 μl each of plasma (immunochemically depleted of factor VII) and 0.25% tissue factor at 37 °C in a total volume of 180 μl, adjusted with TBS containing 1% bovine serum albumin. At the end of 30 min, coagulation was initiated by the addition of 10 μl each of 5 mM $CaCl_2$ and factor VII (1 nM). The time for clot formation was measured using a Fibrometer (BBL Microbiology Systems, Cockeysville, MD).

Inhibition Assays—The inhibition of factor VII function was determined in the two-stage chromogenic assay described above. The peptides were initially screened at a 200 μM final concentration. To determine the concentration of peptide required to inhibit the rate of factor X activation by 50% (CI_{50}), peptides or antipeptide antibody Fab fragments at various concentrations, including buffer (TBS/bovine serum albumin) controls, were incubated with their respective complexes. Following the addition of factor VII, the rate of factor Xa formation was determined, and the percent of the rate of factor X activation in the presence of different concentrations of factor VII peptides relative to buffer controls was calculated. Analogous determinations of CI_{50} values were measured in the specific coagulation assay outlined above. Factor VIIa activity in the presence of varying concentrations of peptide was quantitated by comparing the clotting times to a standard curve constructed from the log of the clot time *versus* the log of a known amount of factor VII in the same reaction mixture without added peptide.

RESULTS

The synthetic peptides representing the binding sites on factor VII and the native protein would be expected to compete for interaction with the components of factor X activation complex (the binding of factor VII to tissue factor or factor X to the assembled enzyme complex) and prevent its activation. On the basis of this rationale, the effect of these peptides on the rate of factor X activation was analyzed in a purified protein system employing a chromogenic assay. To optimize the effect of the peptides, each of the components was titrated, and the concentration of enzyme (factor VII-VIIa) used was in excess relative to the cofactor (tissue factor). For inhibition experiments, the peptides were preincubated with factor X and tissue factor at a concentration of 10^3–10^4-fold molar excess relative to factor VII. A preincubation time

of 30 min was sufficient for the peptides to be in equilibrium with the target proteins, and the rates of factor Xa formation were linear over the 8-min sampling period. The rates of factor Xa formation (Fig. 1) in the presence and absence of synthetic peptides were hyperbolic with respect to factor X (substrate) concentration. The rate of factor Xa formation in the presence of peptide-(285–305) was reduced by ~60%, but by <10% by peptide-(226–243). To further enhance the sensitivity of detection of potential inhibitory peptides, the substrate concentration was maintained at or below the K_m of the reaction, and the enzyme (factor VII) concentration was made as low as practically possible.

The amino acid sequences of 16 synthetic peptides of factor VII and their effect on the rate of factor X activation are depicted in Table I. Each of these peptides was preincubated individually at a final concentration of 200 μM with the components of the factor X activation complex (factor X, tissue factor, and Ca^{2+}) before the addition of factor VII, and the rate of factor Xa formation was determined. Factor X activation was completely inhibited by six of the synthetic peptides: (peptides-(40–50), -(194–204), -(208–229), -(285–305), -(347–361), and (376–390)), and partial inhibition was observed for two peptides (peptides-(170–178) and -(330–340)). Peptides which reduced the rate of factor Xa formation by <25% were considered not significantly inhibitory at this high concentration. None of these inhibitory peptides, except peptide-(347–361), inhibited the amidolytic activity of factor Xa when they were directly incubated with factor Xa, suggesting that only peptide-(347–361) is an active-site inhibitor of factor Xa and that the seven other inhibitory peptides have the ability to prevent the factor VII-mediated activation of factor X.

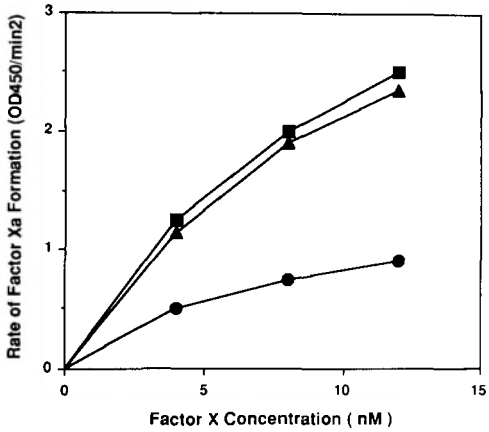

FIG. 1. **Effect of selected synthetic peptides on rate of factor Xa formation as function of concentration of factor X.** A mixture of tissue factor (0.05%), factor X (varying concentrations), and 5 mM $CaCl_2$ was incubated with buffer (■), 248 μM peptide-(226–246) (▲), or 4.5 μM peptide-(285–305) (●). The reaction was started by adding factor VII-VIIa to a final concentration of 0.2 pM. Aliquots were withdrawn at selected time points, and the rate of factor Xa formation was measured using a chromogenic assay as described under "Experimental Procedures."

To determine the relative potency of these inhibitory peptides in preventing the activation of factor X, each of them was incubated individually at varying concentrations with the components of the factor X activation complex before the addition of factor VII, and the rate of factor Xa formation was measured relative to controls lacking the added peptides (Fig. 2). All of the seven inhibitory peptides tested showed a dose-dependent inhibition. However, the relative potency of peptides varied over a wide range. The concentration required for 50% inhibition (CI_{50}) by peptide-(285–305) was 14–22-fold less than that for peptides-(44–50), -(194–214), -(206–229), and -(376–390) and 75–100-fold less than that for peptides-(170–178) and -(330–340). Table II lists the CI_{50} values measured for each peptide. Peptide-(285–305) was the most potent in inhibiting the activation of factor X, whereas peptides-(170–178) and -(330–340) were least effective. The decreasing order of their ability to prevent factor X activation was: 285–305 > 208–229 > 44–50 > 376–390 > 194–214 > 170–178 > 330–340.

To test whether the peptide inhibition of factor X activation observed in a purified protein system could also be demonstrated in a more complex coagulation assay, selected peptides were incubated individually with factor VII-deficient plasma, and clotting activity was determined following the addition of factor VII. The effect of varying concentrations of the three most inhibitory peptides on the clotting activity of factor VII

TABLE I

Effect of synthetic peptides representing primary structure of human factor VII on rate of factor X activation

Peptide	Amino acid sequence	Factor Xa formation[a]
		% of control rate
44–50	YSDGDQC	0
CG + 141–150	(CG)LEKRNASKPQ	100
147–150	SKPQGRIVGGK	100
156–164	GKVCPKGEC	97
170–178	LLVNGAQLC	56
194–214	CFDKIKNWRNLIAVLGEHDLS	5
208–229 + GC	LGEHDLSEHDGDEQSRRVAQVI(GC)	0
226–243 + GC	AQVIIPSTYVPGTTNHDI(GC)	86
246–262	LRLHQPVVLTDHVVPLC	83
262–279	CLPERTFSERTLAFVRFS	NS[b]
285–305 + GC	GQLLDRGATALELMVLNVPRL(GC)	0
303–329	PRLMTQDCLQQSRKVGDSPNITEYMFC	92
330–340	AGYSDGSKDSC	62
347–361 + GC	PHATHYRGTWYLTGI(GC)	0[c]
376–390	VYTRVSQYIEWLQKL	0
387–406	LQKLMRSEPRPGVLLRAPFP	98

[a] The rate of factor Xa formation was determined by an amidolytic assay using purified proteins at a 200 μM concentration of each of the synthetic peptides. Amino acid sequences are numbered according to the primary structure of human factor VII; G and C in parentheses are additional glycyl and cysteinyl residues.

[b] NS, not soluble.

[c] Direct inhibition of factor Xa amidolytic activity.

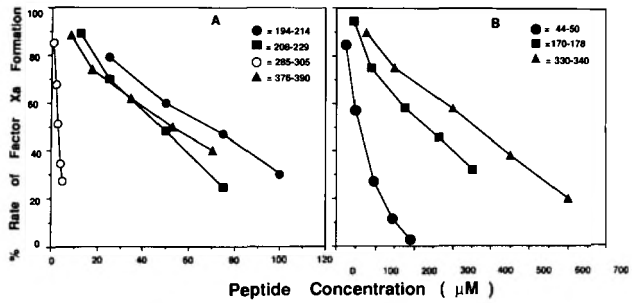

FIG. 2. **Dose-dependent inhibition by factor VII synthetic peptides of rate of factor Xa formation initiated by factor VII-tissue factor complex.** Tissue factor (0.075%) and factor X (12 nM) were combined in the presence of varying concentrations of the indicated peptides and incubated for 30 min at 37 °C. Factor VII (0.2 pM) was added to initiate the reaction. At selected time points, aliquots were removed, and the factor Xa activity was measured. Data are plotted as percentage of the rate of factor Xa formation determined in the absence of competeing peptide *versus* the final concentration of peptides.

TABLE II

Summary of the inhibition of the rate of factor Xa formation and of factor VII coagulant activity by factor VII synthetic peptides

Peptide	Conc for 50% inhibition[a]	
	Factor Xa formation	Coagulant activity
	μM	
44–50	53	ND[b]
170–178	218	ND
194–214	68	ND
208–229	48	225
285–305	3	10
330–340	285	ND
347–361	43	ND
376–390	58	172

[a] The rate of factor Xa formation was determined by amidolytic and coagulation assays as described under "Experimental Procedures" using varying concentrations of each of the inhibiting peptides. The concentration of each peptide which inhibited 50% of the rate of factor Xa formation or factor VII coagulant activity is indicated. The values were calculated relative to those measured in the absence of added peptide.

[b] ND, not determined.

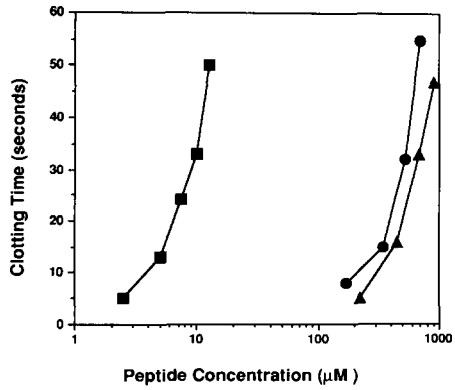

FIG. 3. **Dose-dependent inhibition of factor VII procoagulant activity.** Factor VII-deficient plasma containing tissue factor was incubated with varying concentrations of peptides-(209–221) (●), -(285–305) (■), and -(376–390) (▲) for 30 min at 37 °C. The clotting time was measured after the addition of factor VII and calcium ions. The increase in clotting time relative to that in the absence of competing peptide is plotted against the peptide concentration.

is depicted in Fig. 3. All three peptides tested showed a dose-dependent increase in the clotting time. The CI_{50} values of these three peptides were much higher for factor X activation in the coagulation assay relative to their respective values in the purified protein system (Table II). The 5–10-fold higher CI_{50} value for the coagulation assay suggests that the peptides are more potent inhibitors of factor X activation in a purified system. This may be because of the complexity of the coagulation assay, which involves two additional reaction steps before reaching the end point, and also because of the presence of a higher concentration of factor VII. Furthermore, plasma-derived peptidases or nonspecific adsorption of peptides to plasma proteins may decrease the effective concentration of the synthetic peptides.

To characterize the type of inhibition, the rate of factor Xa formation was determined at different concentrations of factor X while varying the concentration of synthetic peptides. Dixon plots of the data showing the ability of peptides to inhibit the rate of activation of factor X are depicted in Fig. 4. Except for one peptide, the plot of inverse reaction rate *versus* the final concentration of peptide produced linear regression lines which intersected the abscissa, consistent with noncompetitive inhibition. In the case of peptide-(347–361), when the rate of factor Xa formation was determined at different concentrations of chromogenic substrate with varying peptide concentrations, the regression lines intersected at a point above the abscissa, indicating that the type of inhibition is competitive, which is consistent with its ability to inhibit factor Xa amidolytic activity. The peptide inhibition constants (K_i) are summarized in Table III. Peptide-(285–305) was most inhibitory, with a K_i value of 2.4 μM. The K_i values were in the range of 42–65 μM for peptides-(44–50), -(194–214), -(208–229), and -(376–390); and the least inhibitory peptides were at positions 170–178 and 330–340, with K_i values >200 μM.

Finally, to demonstrate further that the inhibitory synthetic peptides represent important regions of factor VII which are responsible for its interaction with proteins involved in the factor X activation complex, polyclonal antibodies were raised against four of these peptides. When antisera were assayed by a solid-phase radioimmunoassay, they bound not only to their respective immunizing peptides, but also to factor VII. The specific antibodies were purified by immunoaffinity chromatograghy on factor VII-agarose. Because the size of the factor VII-IgG complex is three times that of antigen alone, which may cause steric interference in the functional assay. Fab fragments (M_r = 50,000) of specific IgG were prepared and tested for their ability to inhibit the activation of factor X. The effect of varying concentrations of antipeptide Fab fragments on the rate of factor Xa formation is shown in Fig. 5. The Fab fragments specific for each of the four peptides inhibited the rates of factor Xa formation in a dose-dependent manner. The concentration which gives ~50% inhibition ranged from 8 to 25 μM, and the Fab fragment from the antibody raised against peptide-(285–305) was most inhibitory. The fact that antibodies to these inhibitory peptides can react with factor VII supports the notion that the regions which these synthetic peptides represent on factor VII are largely surface-exposed and readily available to interact with tissue factor, factor X, or membrane surfaces, in addition to the antigen-binding sites of the Fab fragments.

DISCUSSION

Macromolecular assembly and protein substrate recognition are important aspects of the complex cascade pathway of blood coagulation. Factor VII-VIIa, in association with tissue

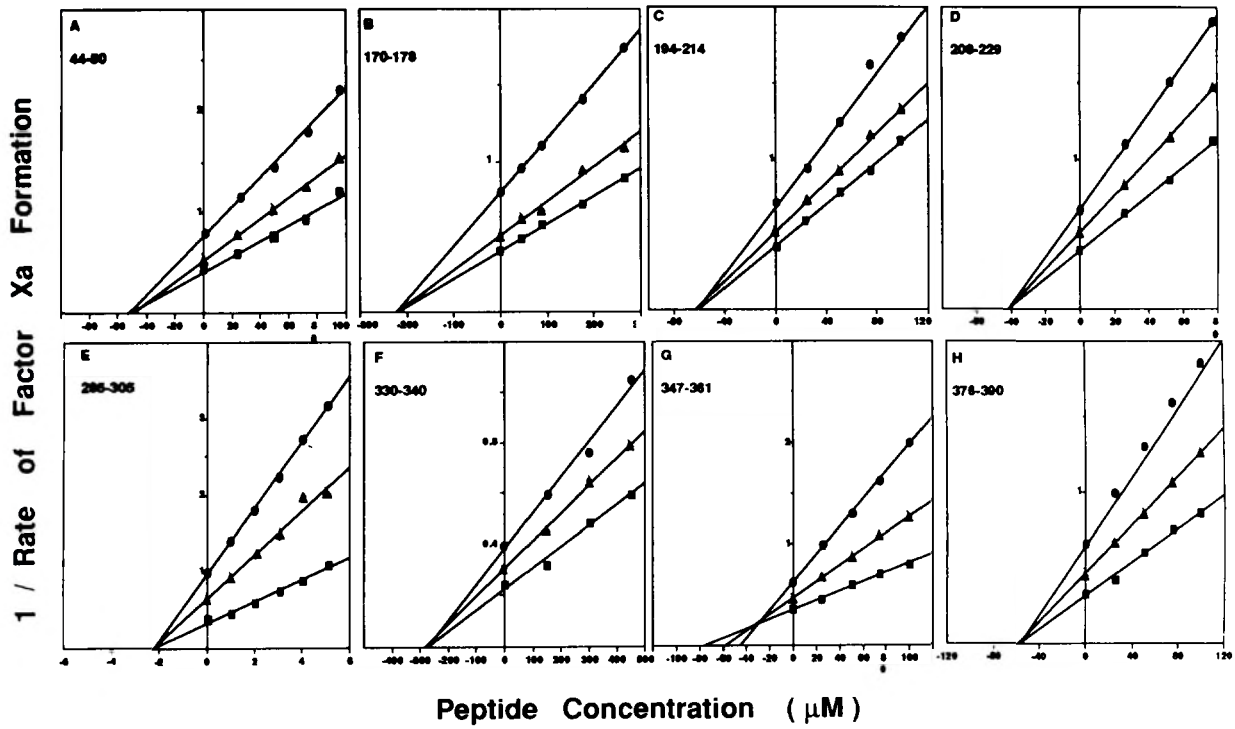

FIG. 4. **Dixon plots of inhibition of rate of activation of factor X by factor VII synthetic peptides.** The indicated peptides at varying concentrations were incubated with factor X at three different concentrations (4, 8, and 12 nM) in the presence of tissue factor (0.075%). After the addition of factor VII (0.2 pM), the rate of factor X formation was determined. The inverse rate of factor Xa formation *versus* the final peptide concentrations is depicted. The point at which the regression lines converge on the abcissa was used to calculate the apparent inhibition constant (K_i) for each peptide.

TABLE III

Peptide inhibition constants of factor VII synthetic peptides for factor X activation

Peptide	K_i[a]
	μM
44–50	54.9 ± 7.2
170–178	215 ± 11.8
194–214	61.1 ± 4.7
208–229	44.3 ± 5.6
285–305	2.8 ± 0.4
330–340	247 ± 13.9
347–361	35.2 ± 6.2
376–390	57.8 ± 8.3

[a] K_i values were determined from Dixon plots of the rate of factor Xa formation and are expressed as the mean ± S.D. for four to five determinations.

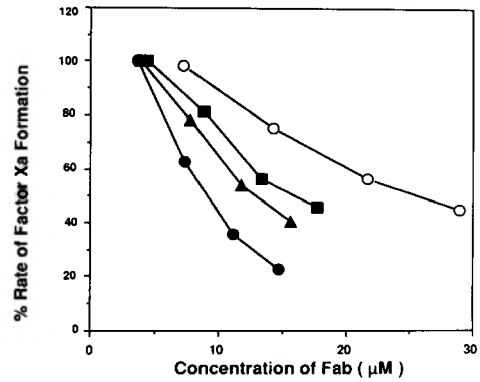

FIG. 5. **Inhibition of factor Xa formation by antibodies raised against selected factor VII synthetic peptides.** Rabbit antibodies raised against peptides-(44–50), (○), -(170–178) (■), -(208–229) (▲), and -(285–305) (●) were affinity-purified on a factor VII-agarose column. The purified Fab fragments were preincubated with factor VII for 15 min at 37 °C. The rate of factor Xa formation relative to the rate observed in the presence of Fab fragments from nonimmune rabbit IgG is plotted *versus* the concentration of each antipeptide Fab fragment.

factor, is the first complex responsible for initiating blood coagulation through the extrinsic pathway. Factor VII shares a substantial degree of primary structural similarity (8) to other vitamin K-dependent serine proteases involved in blood coagulation, such as factors X (40%) and IX (40%), prothrombin (25%), and protein C (40%). Furthermore, a similar level of sequence similarity exists between the catalytic chain of coagulation serine proteases and digestive serine proteases such as trypsin and chymotrypsin (1, 26). Because this class of enzyme appears to have a common tertiary structure, it is reasonable to interpret the structure-function relationship of factor VII using a model based on the conformation of evolutionarily related enzymes (14–18). Sixteen synthetic peptides of factor VII, corresponding to 55% of its primary structure, were employed in this study to identify the molecular sites of interaction. The amino acid sequences of 13 of these peptides correspond to 80% of the catalytic chain, whereas one peptide composes sequences including the activation site, and two peptides represent 10% of the light chain sequence.

The results presented here indicate that 7 of the 16 peptides have the ability to consistently prevent the factor VIIa-tissue factor-mediated conversion of factor X to factor Xa. Competition by these inhibitory peptides with factor VII for interaction with the factor X activation complex was specific since no significant inhibition was observed with the other factor VII peptides. Five of the inhibitory synthetic peptides of factor VII, *i.e.* peptides-(44–50), -(194–214), -(208–229), -(285–305),

and -(376–390), are the most potent inhibitors when compared to the other two, peptides-(170–178) and -(330–340). Hence, the regions represented by the five most potent inhibitory peptides on factor VII may play a more direct role in the assembly of the factor X activation complex. However, additional studies will be required to define the contribution of each individual residue within this region of factor VII. Dixon plots of all the peptides that inhibit the activation of factor X show a noncompetitive type of inhibition, indicating that the peptides do not directly interact with the active site of the activator.

It has been well established that antisera raised against small synthetic peptides are frequently capable of recognizing the native protein (27). Antibodies raised against each inhibitory peptide reacted selectively with the respective peptides and were affinity-purified on an factor VII-agarose column, indicating that the regions represented by these peptides are exposed and are available for interaction with other species at the solvent-accessible surface of factor VII. Amino acid residues of peptides-(44–50), -(208–229), and -(330–340) are predominantly hydrophilic, whereas the residues at positions 285–305 are less so. Although a good correlation between hydrophilicity and surface accessibility has been reported (28) and both indices have been employed in identifying immunogenic peptides (29, 30), not all parts of an epitope need adhere to these criteria. The inhibition of factor X activation by antipeptide IgG Fab fragments further supports the notion that the regions on factor VII represented by these amino acid sequences are largely surface-exposed and mediate its interaction with other molecules, including antigen-binding sites on Fab fragments.

Among seven inhibitory peptides of factor VII, only peptide-(44–50) represents the amino acid sequence of the light chain, and the rest of them correspond to part of the heavy or catalytic chain sequence. The sequence of the light chain inhibitory peptide-(44–50) is located between the Gla domain and the second disulfide loop of the light chain. The tertiary structure of the Gla domain of prothrombin (31, 32) indicates that this region projects off the last turn of the α-helix of the Gla domain and may serve as a scaffold for anchoring a portion of its folded domain. In addition, this region on factors VII, X, and XI is extended by 5 residues and is predicted to be oriented toward the surface. Hence, it is reasonable to speculate that this amino acid sequence on the light chain may play a role in the membrane binding properties of factor VII.

When the primary structures of the catalytic chains of different serine proteases are compared, small stretches of highly conserved sequences occur at various intervals along the polypeptide chain (33). Furie *et al.* (16) identified seven conserved regions (CR) separated by six variable regions (VR) among the coagulation serine proteases. These conserved regions define the internal structure of the catalytic domains and active-site moieties of the serine proteases. The variable regions show little sequence similarity, involve short deletions and insertions, and are thought to be located on the surfaces of the proteins. These regions probably define the molecular surface and may be responsible for their diverse biological properties and selective enzymatic activity. The amino acid sequences of inhibitory peptides-(170–178), -(196–214), -(208–229), -(285–305), and -(330–340) are located in variable regions VR_1, VR_2, VR_3, VR_4, and VR_5 respectively, whereas peptide-(376–390) is in a conserved region (CR_6). It appears that interactions with the factor X activation complex may be mediated by as many as six regions on the solvent-exposed surface of the catalytic or heavy chain of factor VII. However, when the primary structure of the catalytic chain was superimposed on the three-dimensional crystallographic structure of chymotrypsin (34, 35), these inhibitory peptide sequences were found to correspond to only three regions of chymotrypsin. The regions represented by peptides-(170–178), -(196–214), and -(208–229) are spatially close to one another and are located in the extended surface β-loops which lie beyond the active site. Peptide-(285–305) forms a distinct loop located just above the active site. Finally, a region represented by peptides-(330–340) and -(376–390) is located directly beneath the extended substrate-binding pocket of chymotrypsin, which is lined by Ser^{214}-Trp^{215}-Gly^{216}. The peptide backbone of these residues is thought to interact with the side chain of the substrate to properly orient the bond that is to be cleaved (36). Since peptides corresponding to these three regions, which are arranged spatially apart from one another on the surface of the molecule, inhibited factor X activation, they could serve as specific molecular sites of interaction with the components of the extrinsic pathway activation complex. In conclusion, the assembly and function of the extrinsic pathway complex are mediated by at least four molecular loci on factor VII. However, identification of which region(s) on factor VII interact with complementary components such as tissue factor, factor X, and the membrane surface of the extrinsic pathway complex will require further investigation.

Acknowledgments—We would like to thank Drs. Harold L. James, James H. Morrissey, and Michael K. Pangburn for critical review of the manuscript and Paula Roberts for her excellent technical assistance in the isolation and characterization of the synthetic peptides.

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