facilitate the molecule's attachment to the cell's membrane. Thus, this mutation may accelerate amyloid deposition in the cerebral vessel walls.

Hereditary cerebral hemorrhage with amyloidosis described in Icelandic patients (HCHWA-I) is histopathologically and clinically similar to HCHWA-D (*19*). The brains of HCHWA-I patients demonstrate amyloid angiopathy; however, unlike HCHWA-D, the brain parenchyma is not affected, and the amyloid fibrils are derived from degradation of a variant of cystatin C, an inhibitor of cysteine proteinases (*20*). The variant cystatin C gene contains a point mutation that results in a single amino acid substitution (glutamine instead of leucine) (*21, 22*). Therefore, the amyloid precursor proteins in two inherited forms of cerebral amyloid angiopathies, HCHWA-D and HCHWA-I, are protease inhibitors that may be present in the circulation (*11, 21, 23, 24*) and have a substitution in their respective genes that gives rise to the same amino acid. The relation between the presence of glutamine in these proteins and the formation and deposition of amyloid fibrils in cerebral vessel walls is unknown.


REFERENCES AND NOTES

1. A. R. Wattendorff, G. Th. A. M. Bots, L. N. Went, L. J. Endtz, *J. Neurol. Sci.* **55**, 121 (1982); W. Luyendijk, G. Th. A. M. Bots, M. Vegter-van Der Vlis, L. N. Went, *Ned. Tijdschr. Geneeskd.* **130**, 1935 (1986).
2. S. G. van Duinen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5991 (1987).
3. F. Coria, E. M. Castaño, B. Frangione, *Am. J. Pathol.* **129**, 422 (1987).
4. G. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun.* **120**, 885 (1984); *ibid.* **122**, 1131 (1984).
5. C. L. Masters *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245 (1985).
6. C. W. Wong, V. Quaranta, G. G. Glenner, *ibid.*, p. 8729.
7. F. Prelli *et al.*, *Biochem. Biophys. Res. Commun.* **151**, 1150 (1988); F. Prelli, E. M. Castaño, G. G. Glenner, B. Frangione, *J. Neurochem.* **51**, 648 (1988).
8. J. Kang *et al.*, *Nature* **325**, 733 (1987).
9. D. Goldgaber, M. I. Lerman, O. W. McBride, U. Saffiotti, D. C. Gajdusek, *Science* **235**, 877 (1987); R. E. Tanzi *et al.*, *ibid.*, p. 880; N. K. Robakis, N. Ramakrishna, G. Wolfe, H. M. Wisniewski, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4190 (1987).
10. N. Kitaguchi, Y. Takahashi, Y. Tokushima, S. Shiojiri, H. Ito, *Nature* **331**, 530 (1988).
11. T. Oltersdorf *et al.*, *ibid.* **341**, 144 (1989); W. E. Van Nostrand *et al.*, *ibid.*, p. 546.
12. H. G. Lemaire *et al.*, *Nucleic Acids Res.* **17**, 517 (1989).
13. M. P. Vitek *et al.*, *Mol. Brain Res.* **4**, 121 (1988).
14. P. H. St George-Hyslop *et al.*, *Science* **235**, 885 (1987).
15. R. E. Tanzi *et al.*, *Nature* **329**, 156 (1987); C. Van Broeckhoven *et al.*, *ibid.*, p. 153; G. D. Schellenberg *et al.*, *Science* **241**, 1507 (1988).
16. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
17. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
18. F. Prelli *et al.*, in preparation.
19. G. Gudmundsson, J. Hallgrimsson, T. A. Jonasson, O. Bjarnason, *Brain* **95**, 387 (1972).
20. D. H. Cohen, H. Feiner, O. Jensson, B. Frangione, *J. Exp. Med.* **158**, 623 (1983).
21. J. Ghiso, O. Jensson, B. Frangione, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2974 (1986).
22. E. Levy *et al.*, *J. Exp. Med.* **169**, 1771 (1989).
23. B. Rumble *et al.*, *N. Engl. J. Med.* **320**, 1446 (1989); D. J. Selkoe *et al.*, *J. Neuropathol. Exp. Neurol.* **48**, 377 (1989).
24. J. Ghiso, F. Tagliavini, W. F. Timmers, B. Frangione, *Biochem. Biophys. Res. Commun.* **163**, 430 (1989); M. R. Palmert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6338 (1989).


25. We thank F. Hitchcock for manuscript preparation. Supported by the Alzheimer's Disease and Related Disorders Association, Inc., grant IIRG-89-112 (to E.L.), and NIH grant AG 05891 (to B.F.).

12 January 1990; accepted 25 April 1990


# Platelet Coagulation Factor $XI_a$-Inhibitor, a Form of Alzheimer Amyloid Precursor Protein


RAYMOND P. SMITH, DARRYL A. HIGUCHI, GEORGE J. BROZE, JR.*



**An inhibitor of coagulation factor $XI_a$ was purified from serum-free conditioned medium of HepG2 liver cells. Platelets stimulated with thrombin or calcium ionophore (A23187) secrete a protein functionally and immunologically identical to the inhibitor, implying a role for this inhibitor in hemostasis. Analysis of the amino-terminal amino acid sequence and immunologic reactivity showed the inhibitor to be a truncated form of the Alzheimer's amyloid precursor protein that contains a Kunitz-type serine protease inhibitor domain and at least a portion of the amyloid β protein. It inhibits factor $XI_a$ and trypsin with a $K_i$ of 450 ± 50 pM and 20 ± 10 pM, respectively. Heparin (1 unit/ml) did not significantly effect inhibition of trypsin, but inhibition of $XI_a$ was 15 times greater ($K_i$ = 25 ± 15 pM) in the presence of heparin.**


COAGULATION FACTOR XI CIRCULATES in plasma as the zymogen of a serine protease. Individuals deficient in factor XI suffer a mild to moderate bleeding diathesis characterized by mucosal and post-operative bleeding (*1*). Factor XI is activated by limited proteolytic cleavage through the action of the contact factors (factor $XII_a$, prekallikrein, and high molecular weight kininogen). Activated factor XI ($XI_a$) is a protease that consists of two identical disulfide-bonded subunits, each with a catalytic site, and functions in coagulation as an activator of factor IX. Platelets stimulated by thrombin or calcium ionophore (A23187) release a protein that reversibly inhibits $XI_a$ but does not form $XI_a$-inhibitor complexes that are stable to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (*2, 3*).

Lysed platelets and the supernatant of A23187-treated platelets contain a protein of ~112,000 kD that binds [$^{125}$I]-$XI_a$ (Fig. 1A). The serum-free conditioned medium of human hepatoma cells (HepG2) contains the same moiety (Fig. 1A). Approximately 1.3 mg of purified inhibitor (*4*) was recovered from 90 liters of serum-free conditioned medium. SDS-PAGE shows a predominant band at 112 kD (120 kD when reduced) with additional faint bands at 94, 66.5, and 55 kD (Fig. 1B). Antibodies to the purified factor $XI_a$-inhibitor ($XI_a$I) from HepG2 medium adsorbed the inhibitor released from platelets; thus, the HepG2 and platelet proteins are related antigenically (Fig. 2A).

The $NH_2$-terminal amino acid sequence of the 112-kD protein is X E V P T D G N A G L L A E P Q (*5*), which matches exactly residues 19 through 35 of the amino acid sequence predicted from the amyloid precursor protein cDNA sequence (*6–8*). When trypsin (rather than anhydrotrypsin) affinity chromatography was used in the purification, the minor bands present in Fig. 1B were considerably more abundant. The $NH_2$-terminal sequences determined from each band are identical to that of the 112-kD protein, suggesting that the smaller peptides resulted from proteolysis at the COOH-terminus, which may have occurred during the affinity chromatography.

A prominent protein found in the brains of patients with Alzheimer's disease is the amyloid β protein (amyloid A4 protein) (*9, 10*). The amyloid β protein represents a small part of a transmembrane amyloid precursor protein (APP) that, through alternative mRNA splicing, is produced in at least three different forms (*6–8*). The two longer proteins ($APP_{751}$ and $APP_{770}$) contain a Kunitz-type serine protease inhibitor domain, whereas this domain is lacking in the shorter form containing 695 amino acids


Division of Hematology/Oncology, Department of Medicine, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110.

*To whom correspondence should be addressed.

($APP_{695}$) (*6–8*). An mRNA encoding a putative secreted form of the precursor protein was identified that contains the Kunitz domain, but lacks the COOH-terminal amyloid, transmembrane, and cytoplasmic domains (*11*). Protease nexin II, a proteinase inhibitor, was also identified as a form of the APP (*12, 13*).

Antibodies to several synthetic peptides that represent domains of the APP (*14, 15, 16, 17*) recognized both the purified $XI_aI$ (Fig. 2B) and the inhibitor in the supernatant of activated platelets (Fig. 2C). Antisera to the amyloid domain but not to the COOH-terminal domain of the APP reacted with $XI_aI$, consistent with the hypothesis that $XI_aI$ represents a proteolytically truncated form of the transmembrane APP (*17*).

Kinetic experiments show that protease inhibition by $XI_aI$ is of the slow, tight-binding, reversible type (Fig. 3, A and B), which is typical of Kunitz-type inhibitors (*18*). When identifying its inhibitory spectrum with various test enzymes at 0.50 nM and an eight- to tenfold excess of inhibitor, the purified $XI_aI$ did not significantly affect the activities of thrombin, factor $VII_a$, the $VII_a$-tissue factor complex, factor $X_a$, factor $XII_a$, tissue plasminogen activator, urokinase, kallikrein, or leukocyte elastase. Chymotrypsin, however, was inhibited ~25%, and $XI_a$ and trypsin were inhibited more than 90%. The stoichiometry of binding of the inhibitor to $XI_a$ appeared to be 1.8:1 (0.9 molecules inhibitor per $XI_a$ active site), and 1:1 with trypsin (Fig. 3C). The $K_i$ of $XI_aI$ was determined to be 450 ± 50 pM for $XI_a$ and 20 ± 10 pM for trypsin (*19*). Whereas heparin (1 unit/ml) has little effect on the trypsin:$XI_aI$ interaction ($K_i$ = 20 ± 10 pM), it enhances $XI_aI$ inhibition of $XI_a$ ($K_i$ = 25 ± 15 pM). This effect of heparin

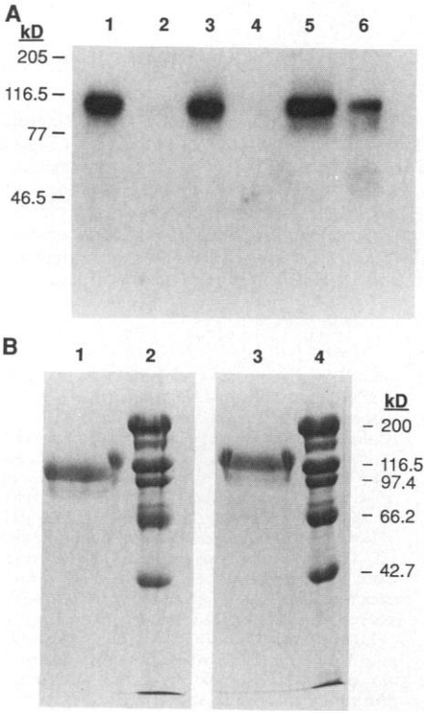


**Fig. 1.** (**A**) Ligand blot of factor $XI_a$ inhibitor. Lane 1, lysed, unstimulated platelets. Lane 2, supernatant of unstimulated platelets. Lane 3, supernatant of platelets activated with calcium ionophore A23187. Lane 4, platelet pellet after activation. Lane 5, concentrated HepG2 media. Lane 6, 125 ng of purified HepG2 $XI_aI$. Isolated platelets (*23*) were suspended in TSD (0.1 M NaCl, 0.05 M tris-HCl, pH 7.5, 1% dextrose) at $10^9$ platelets per milliliter and stimulated with 1 μM A23187 for 10 min. Platelets were separated from activated supernatant by centrifugation through oil (*24*). Ligand blotting was performed (*25*) with [$^{125}$I]$XI_a$ as the probe. Whole platelets were lysed with 1% SDS. HepG2 concentrated serum-free media was obtained after cadmium chloride precipitation and elution (*4*). Relative molecular sizes are shown on the left in kilodaltons. (**B**) SDS–polyacrylamide gel electrophoresis of purified $XI_aI$. Lanes 1 and 3, 15 μg of purified inhibitor. Lanes 2 and 4, protein standards. Samples in lanes 1 and 2 are unreduced, samples in lanes 3 and 4 were reduced with 5% β-mercaptoethanol. SDS-PAGE was performed by the method of Laemmli (*26*), and proteins were stained with Coomassie Brilliant Blue R-250. Relative molecular sizes are shown on the left in kilodaltons.

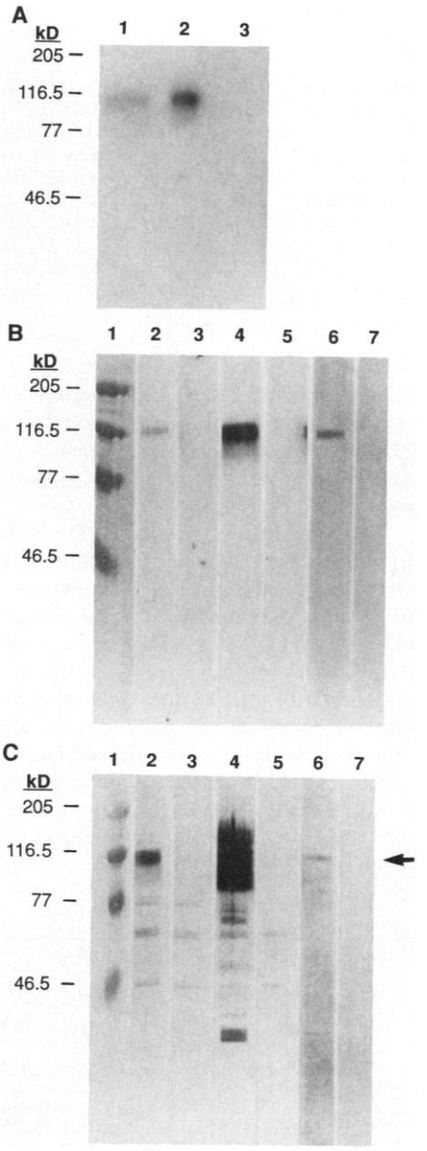


**Fig. 2.** Immunoabsorption and immunoblot analysis of purified factor $XI_a$ inhibitor and activated platelet supernatant. Platelets ($1 \times 10^9$ per milliliter) were stimulated with thrombin (1 unit/ml) in the presence of 10 m*M* EDTA and their releasate isolated as in Fig. 1. (**A**) Rabbits were immunized with purified $XI_aI$ from HepG2 cells (*4*) and protein A–purified immunoglobulin G (IgG) was then immobilized on Affigel-10 according to the manufacturer's instructions (Bio-Rad, Richmond, California) at a concentration of 5 mg/ml. Mixtures of platelet releasate (400 μl) and IgG-Affigel beads (100 μl) were rocked for 60 min at room temperature, the beads washed extensively with 0.1 M NaCl, 0.05 M tris-HCl, pH 7.5, and the immunoadsorbed proteins eluted from the beads by adding 60 μl of 3% SDS. Samples (25 μl) were subjected to SDS-PAGE and ligand blotting was performed using [$^{125}$I]trypsin as the probe. Lane 1, activated platelet supernatant. Lane 2, anti-$XI_aI$ Affigel eluate. Lane 3, anti-factor VII Affigel eluate (*27*) (as a control for nonspecific adsorption). Relative molecular sizes are shown on the left in kilodaltons. (**B** and **C**) Rabbit antibodies against peptides representing specific regions of $APP_{751}$ were used to further characterize the $XI_aI$. Reduced samples of purified inhibitor (B) and inhibitor partially purified from platelet releasate by heparin agarose affinity chromatography (*28*) (C) were subjected to SDS-PAGE and transferred to nitrocellulose paper. After blocking with 3% non-fat milk, the blots were incubated with appropriate dilutions of a specific antisera and developed using alkaline phosphatase coupled goat anti-rabbit IgG (Sigma, St. Louis, Missouri). Lane 1, prestained molecular weight standards. Lane 2, antisera to amino acids 284 to 299 (Kunitz domain) (*14*). Lane 3, antisera to amino acids 284 to 299 after absorption with 50 μg of the specific peptide (*14*). Lane 4, antisera to amino acids 45 to 62 (*15*). Lane 5, antisera to amino acids 732 to 751 (COOH-terminus) (*16*). Lane 6, antisera to amino acids 653 to 692 (amyloid β protein) (*17*). Lane 7, antisera to the amyloid β protein after absorption with 50 μg representing the first 28 amino acids of the amyloid β protein (amino acids 653 to 680) (Biochem, California). Relative molecular sizes are shown at the left. In (C) the arrow indicates the position of $XI_aI$. The staining of several lower molecular weight bands by the antisera to the peptide 284-299 is apparently nonspecific since it is not affected by pre-absorption of the antisera with this peptide (compare lanes 2 and 3). The specific peptide was not available for absorption of the antisera to amino acids 45–62 (lane 4). Several of the additional bands present correlate with the nonspecific staining seen in lanes 3 and 5. Other bands could also represent nonspecific binding or conceivably additional forms of the APP or degradation products of $XI_aI$ that do not contain the Kunitz domain [due to lack of [$^{125}$I]$XI_a$ binding (Fig. 1)]. Staining of two lower molecular size bands by the antisera to peptide 732-751 (COOH-terminus) (lane 5) remained following pre-absorption of the antisera with this peptide.

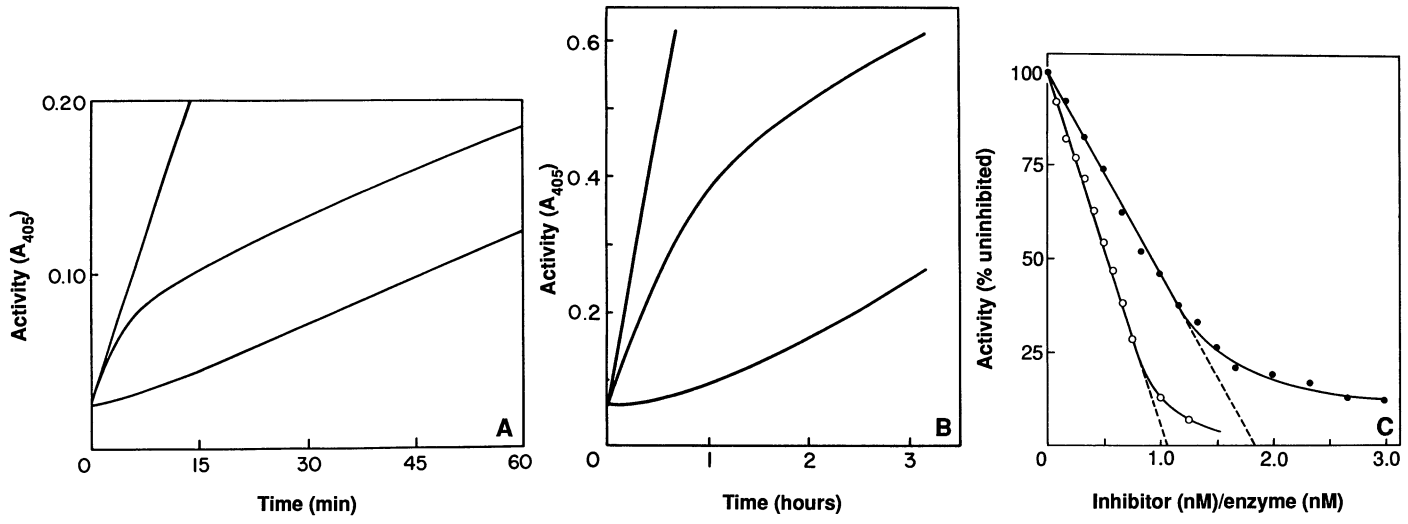


**Fig. 3.** Kinetics and stoichiometry of the $XI_aI$. (**A** and **B**) Slow, tight-binding, reversible inhibition of $XI_a$ and trypsin by $XI_aI$. In both (A) and (B) the tracings represent: top, cleavage of substrate by enzyme in absence of inhibitor; middle, cleavage of substrate by enzyme added to mixture of substrate and inhibitor; bottom, cleavage after addition of substrate to preincubated (1 hour, 21°C) mixture of enzyme and inhibitor. Substrates were the chromogens S2366 and Spectrazyme $X_a$. Product generation was measured by absorbance at 405 nm. (A) $XI_a$ (0.25 nM), pryoGlu-Pro-Arg-pNA (S2366) (0.3 mM) and $XI_aI$ (3 nM). (B) Trypsin (0.5 nM), Spectrazyme $X_a$ (1 mM), and $XI_aI$ (4.2 nM). (**C**) Stoichiometry of $XI_aI$ binding to $XI_a$ or trypsin. $XI_a$ (● − ●), or trypsin (○ − ○), each at 1 nM, were incubated without $XI_aI$ or with various concentrations of the $XI_aI$ for 1 hour at 21°C in 900 μl of TBSA. Substrates (S2366 and Spectrozyme $XI_a$, respectively) were then added and the initial rates of product generation were measured by absorbance at 405 nm. The concentration of trypsin was determined by active site titration using *p*-nitrophenyl *p'*-guanidinobenzoate (*29*); concentrations of $XI_a$ and $XI_aI$ were derived from $A_{280}$ measurements using extinction coefficients of 13.4 and 5.7, respectively (*4*). Lines were drawn by inspection.

may be physiologically important, because sulfated glycosaminoglycans are prominent constituents of the plaques characteristic of Alzheimer's disease and are located at the surface of several cell types, in particular endothelial cells (*20, 21*).

The role, if any, of the APP Kunitz protease inhibitor domain in the pathogenesis of Alzheimer's disease is not known. It is conceivable that inhibition of an enzyme other than $XI_a$ is of importance in this regard. However, the presence of the APP containing the Kunitz domain on the surface of a variety of cells (*22*) and the release of a truncated form by stimulated platelets, which are at high concentrations at the site of a developing thrombus, suggest that the APP may play a role in the regulation of coagulation.

## REFERENCES AND NOTES


1. A. H. Schmaier, M. Silverberg, A. P. Kaplan, R. W. Colman, in *Hemostasis and Thrombosis*, R. W. Colman, J. Hirsh, V. J. Marder, E. W. Salzman, Eds. (Lippincott, Philadelphia, 1987), pp. 18–38.
2. H. Soons, T. Janssen-Claessen, H. C. Hemker, G. Tans, *Blood* **68**, 140 (1986).
3. P. N. Walsh, D. Sinha, F. Kueppers, F. S. Seaman, K. B. Blankstein, *J. Clin. Invest.* **80**, 1578 (1987).
4. Purification of $XI_aI$. The serum-free conditioned medium (90L) of HepG2 cells was harvested, precipitated with $CdCl_2$, and the supernatant passed through a bovine factor $X_a$-Affigel affinity column to remove lipoprotein-associated coagulation inhibitor as described [G. J. Broze, Jr., L. A. Warren, J. J. Girard, J. P. Miletich, *Thromb. Res.* **48**, 253 (1987)]. The preparation was then diluted with water to a conductivity of 18 mmho (pH 8.0) and applied to a 2.5 by 30 cm column of Q-Sepharose. The column was developed with a gradient from 0.15 to 1.0 M NaCl in 0.05 M tris-HCl, pH 8.0, and fractions containing $XI_aI$ activity (eluting at 22 mmho) were pooled. This sample was applied to a 10-ml column of anhydrotrypsin agarose [prepared as described by S. Ishii, H. Yokosawa, T. Kumakazi, I. Nakamura, *Methods Enzymol.* **91**, 378 (1983)] and eluted with 1 M arginine, pH 5.7. After dialysis against 0.1 M NaCl, 0.05 M tris-HCl, pH 7.5, the purified protein was stored at −70°C. Its concentration was determined by measurement at $A_{280}$; the extinction coefficient was assumed to be 5.7 on the basis of the predicted amino acid composition of the secreted form of the amyloid precursor protein (*22*) [H. Edelhoch, *Biochemistry* **6**, 1948 (1967)].
5. $NH_2$-terminal sequencing was performed with ~25 μg of purified $XI_aI$ after SDS-PAGE and electroblotting onto a polyvinylidene difluoride membrane as described [W. F. Novotny, T. J. Girard, J. P. Miletich, G. J. Broze, Jr., *J. Biol. Chem.* **264**, 18832 (1989)]. Single letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and X, indeterminant.
6. J. Kang *et al.*, *Nature* **325**, 733 (1987).
7. P. Ponte *et al.*, *ibid.* **331**, 525 (1988).
8. N. Kitaguchi, Y. Takahashi, Y. Tokushima, S. Shiojiri, H. Ito, *ibid.* p. 530.
9. C. L. Masters *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245 (1985).
10. D. J. Selkoe, C. R. Abraham, M. B. Podlisny, L. K. Duffy, *J. Neurochem.* **146**, 1920 (1986).
11. F. de Sauvage and J.-N. Octave, *Science* **245**, 651 (1989).
12. T. Oltersdorf *et al.*, *Nature* **341**, 144 (1989).
13. W. E. Van Nostrand *et al.*, *ibid.*, p. 546.
14. M. R. Palmert *et al.*, *Biochem. Biophys. Res. Commun.* **165**, 182 (1989).
15. M. R. Palmert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6338 (1989).
16. D. J. Selkoe *et al.*, *ibid.* **85**, 7341 (1988).
17. C. L. Joachim, L. K. Duffy, J. H. Morris, D. J. Selkoe, *Brain Res.* **474**, 100 (1988).
18. E. Antonini, P. Ascenzi, E. Menegatti, M. Guarneri, *Biopolymers* **22**, 363 (1983).
19. Determination of $K_i$. Constant concentrations of trypsin (0.5 nM) or $XI_a$ (0.25 nM = 0.5 nM active sites) and $XI_aI$ (6.25 nM) with or without heparin (1 u/ml) were incubated 1 hour at 21°C in 900 μl of TBSA (0.1 M NaCl, 0.05 M tris-HCl, pH 7.5, bovine serum albumin at 1 mg/ml). Various concentrations of a chromogenic substrate (Spectrozyme $X_a$ for trypsin; pryoGlu-Pro-Arg-pNA, S2366, for $XI_a$) in 100 μl were then added and rates of generation of product were followed by absorbance at $A_{405}$ until they became linear. This required ~45 min for the $XI_a$-$XI_aI$ reactions and ~4 hours for the $XI_a$-$XI_aI$-heparin reactions and the trypsin-$XI_aI$ reactions with or without heparin (Fig. 3). A double reciprocal plot of $\Delta$ $(A_{405}/\text{min})^{-1}$ versus (substrate concentration)$^{-1}$ was used to determine $K_{apparent}$ ($K_{app}$). $K_i$ was calculated from the relationship $K_{app} = K_m (1 + [I]/K_i)$ where [I] is the concentration of $XI_aI$ and $K_m$ is the Michaelis constant of the particular chromogenic substrate for the enzyme. $K_m$ values were determined independently in the presence and absence of heparin. $K_i$ values (mean ± standard deviations) were calculated from three separate experiments.
20. M. Hook, L. Kjellen, S. Johansson, J. Robinson, *Annu. Rev. Biochem.* **53**, 847 (1984).
21. A. D. Snow *et al.*, *Hema. Pathol.* **18**, 506 (1987).
22. A. Weideman *et al.*, *Cell* **57**, 115 (1989).
23. D. M. Tollefsen, J. R. Feagler, P. W. Majerus, *J. Clin. Invest.* **53**, 211 (1974).
24. J. P. Miletich, C. M. Jackson, P. W. Majerus, *J. Biol. Chem.* **253**, 6908 (1978).
25. G. J. Broze, Jr., and J. P. Miletich, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1886 (1987).
26. M. K. Laemmli, *Nature* **227**, 680 (1970).
27. G. J. Broze, Jr., S. Hickman, J. P. Miletich, *J. Clin. Invest.* **76**, 937 (1985).
28. The supernatant of activated platelets was obtained as described above. Supernatant (3 ml) was passed over a heparin agarose II column (Sigma, St. Louis, MO) equilibrated in 0.05 M NaCl, 0.025 M tris-HCl, pH 7.5, and eluted with 1 M NaCl, 0.05 M tris-HCl, pH 7.5. The eluate was concentrated by centrifugation through a Centricon-30 microconcentrator (Amicon, Danvers, MA) to ~200 μl.
29. T. Chase, Jr., and E. Shaw, *Method in Enzymol.* **19**, 20 (1970).


30. We thank D. Selkoe and S. Younkin for providing antisera to specific peptides of the amyloid precursor protein.

19 December 1989; accepted 27 April 1990