Inactivation of Factor XII Active Fragment in Normal Plasma

Predominant Role of C$\bar{1}$-Inhibitor

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Abstract. To define the factors responsible for the inactivation of the active fragment derived from Factor XII (Factor XIIf) in plasma, we studied the inactivation kinetics of Factor XIIf in various purified and plasma mixtures. We also analyzed the formation of ^{125}I-Factor XIIf-inhibitor complexes by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In purified systems, the bimolecular rate constants for the reactions of Factor XIIf with C$\bar{1}$-inhibitor, α_2-antiplasmin, and antithrombin III were 18.5, 0.91, and 0.32×10^4 M^{-1} min^{-1}, respectively. Furthermore, SDS-PAGE analysis revealed that 1:1 stoichiometric complexes were formed between ^{125}I-Factor XIIf and each of these three inhibitors. In contrast, kinetic and SDS-PAGE studies indicated that Factor XIIf did not react with α_1-antitrypsin or α_2-macroglobulin.

The inactivation rate constant of Factor XIIf by prekallikrein-deficient plasma was 14.4×10^{-2} min^{-1}, a value that was essentially identical to the value predicted from the studies in purified systems (15.5×10^{-2} min^{-1}). This constant was reduced to 1.8×10^{-2} min^{-1} when Factor XIIf was inactivated by prekallikrein-deficient plasma that had been immunodepleted (<5%) of C$\bar{1}$-inhibitor. In addition, after inactivation in normal plasma, 74% of the active ^{125}I-Factor XIIf was found to form a complex with C$\bar{1}$-inhibitor, whereas 26% of the enzyme formed complexes with α_2-antiplasmin and antithrombin III. Furthermore, 42% of the labeled enzyme was still complexed with C$\bar{1}$-inhibitor when ^{125}I-Factor XII was inactivated in hereditary angioedema plasma that contained 32% of functional C$\bar{1}$-inhibitor. This study quantitatively demonstrates the dominant role of C$\bar{1}$-inhibitor in the inactivation of Factor XIIf in the plasma milieu.

Introduction

The active fragment derived from Factor XII (Factor XIIf)[1] is a serine protease (M_r 28,000) that results from proteolytic cleavage of Factor XII. This cleavage can occur on a surface, during contact activation of normal plasma (1-3), or in solution, as a consequence of Factor XII digestion by various proteolytic enzymes, including plasmin and plasma kallikrein (3-5). In vitro, Factor XIIf is a potent liquid-phase activator of plasma prekallikrein (6-7), which also activates Factor VII (8), plasminogen (9), and the first component of the classical pathway of complement (10, 11). However, Factor XIIf exhibits minimal clot-promoting activity (6, 7). In vivo, severe arterial hypotension was observed after the administration to surgical patients of plasma protein fraction containing Factor XIIf (12). This observation suggested that the circulatory collapse seen in these patients depended upon Factor XIIf-mediated plasma prekallikrein activation (12). Direct support for this suggestion was recently obtained by the observation that increased plasma bradykinin levels were observed in patients who presented arterial hypotension as a result of the administration of plasma protein fraction that contained Factor XIIf (13).

Studies in purified systems have shown that Factor XIIf is

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[1] *Abbreviations used in this paper:* DFP, diisopropylfluorophosphate; Factor XIIf, active fragment derived from Factor XII; p-NPGB, *p*-nitrophenyl p′-guanidinobenzoate HCl; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

inactivated by several plasma protease inhibitors, including C$\bar{1}$-inhibitor, antithrombin III, and α_2-antiplasmin (14–18). Immunochemical studies have indicated that the incubation of Factor XIIf with normal plasma resulted in the formation of a complex involving Factor XIIf and C$\bar{1}$-inhibitor (19). Although these investigations demonstrate that at least three plasma protease inhibitors can inactivate Factor XIIf, they do not provide a quantitative description of the factors responsible for Factor XIIf inactivation in the plasma milieu. Such an analysis is reported in this manuscript.

Methods

Materials. Dextran sulfate, CNBr-activated Sepharose 4B, quaternary aminoethyl-Sephadex A-50, Sephadex G-25 and G-75, and heparin-Sepharose CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden); trypsin-N-tosyl-L-phenylalanine chloromethyl ketone and soybean trypsin inhibitor (Millipore Corp., Freehold, NJ); H-D-Pro-Phe-Arg-*p*-nitroanilide (S-2302) (Kabi Diagnostica, Stockholm); *p*-nitrophenyl p′-guanidinobenzoate HCl (*p*-NPGB) (Merck and Co., Inc., Darmstadt, West Germany); diisopropylfluorophosphate (DFP) (Fluka AG, Buchs, Switzerland); Bolton-Hunter reagent (Amersham Corp., Amersham, England); antiserum to C$\bar{1}$-inhibitor and antithrombin III (Behringwerke AG, Marburg, West Germany) and to α_2-antiplasmin (Nordic Immunological Laboratories, Tilburg, The Netherlands) were purchased from the designated supplier.

Fresh plasma anticoagulated with citrate phosphate dextrose adenine (CPDA-1; Baxter Travenol Laboratories, Castlebar, Ireland) and generously supplied by the Geneva Blood Center (Dr. P. A. Miescher) was used for protein purification. All other plasma samples were prepared by adding 9 vol blood to 1 vol 0.11 M sodium citrate. Citrated blood was then centrifuged at 3,000 *g* for 15 min at 4°C. Thereafter, plasma samples were kept frozen at −70°C until use. The reference plasma pool was obtained by mixing plasma from 80 healthy blood donors (a gift from Dr. F. Bachmann, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland). Plasma partially deficient in C$\bar{1}$-inhibitor was obtained from an individual with classical hereditary angioedema. Functional and antigenic levels of C$\bar{1}$-inhibitor in this plasma were, respectively, 32 and 13% of the levels measured in the reference plasma pool (20). For some experiments, hereditary angioedema plasma was pretreated with an antiserum to C$\bar{1}$-inhibitor at a concentration known to immunoprecipitate all the C$\bar{1}$-inhibitor contained in the same volume of normal plasma. Prekallikrein-deficient plasma was obtained from Dr. C. F. Abildgaard (University of California, Davis, Medical Center, Sacramento, CA). The levels of both functional and antigenic C$\bar{1}$-inhibitor in prekallikrein-deficient plasma were 140% of the levels measured in the reference plasma pool. For some kinetic studies, prekallikrein-deficient plasma was depleted of C$\bar{1}$-inhibitor by immunoaffinity chromatography on CNBr-activated Sepharose 4B to which immunopurified antibodies to C$\bar{1}$-inhibitor had been covalently linked (21). In this plasma, C$\bar{1}$-inhibitor, antithrombin III, and α_2-antiplasmin were, respectively, <5, 54, and 74% of the levels observed in native prekallikrein-deficient plasma, as assessed by radial immunodiffusion (22). Furthermore, this plasma contained <5% of functional C$\bar{1}$-inhibitor (20). α_2-Antiplasmin-depleted plasma was prepared by immunoadsorption of normal plasma with purified anti-α_2-antiplasmin antibodies covalently coupled to an agarose matrix (23). This plasma contained <2% of the α_2-antiplasmin level of normal plasma. For some experiments, normal plasma and α_2-antiplasmin-depleted plasma were made deficient of antithrombin III by affinity chromatography on heparin-Sepharose. This treatment resulted in a >80% reduction of immunoreactive antithrombin III, as assessed by double-diffusion analysis (22).

Preparation of proteins. Factor XIIf was prepared using a modification of a previously described method (24). Plasma (210 ml) was activated by the addition of acetone (53 ml) and dextran sulfate (65 mg) for 30 min at 23°C. This mixture was dialyzed for 16 h against tap water and centrifuged at 10^4 *g* for 15 min at 4°C. The supernatant was collected and its pH was adjusted to 8.0 with 0.5 M Tris-base. The activated plasma was applied to a QAE Sephadex A-50 column (2.5 × 75 cm) equilibrated with 50 mM Tris–HCl, pH 8.0. After extensive washing with the starting buffer, a gradient of 2,500 ml to a limit of 0.6 M NaCl in the same buffer was applied. The fractions whose conductivity ranged from 11 to 17 mmho exhibited prekallikrein-activating activity when assayed on $CHCl_3$-treated plasma (25). These fractions were concentrated in an ultrafiltration unit (Amicon Corp., Scientific Systems Div., Danvers, MA) by using a PM10 membrane. The concentrated material was gel filtered on a Sephadex G-25 column (5 × 90 cm), equilibrated with 10 mM ammonium acetate, pH 7.0, and lyophilized. This material was dissolved in 20 mM Tris–HCl, pH 8.0, and gel filtered on a Sephadex G-75 column (2.5 × 84 cm) equilibrated in the same buffer. The last step of the purification was a gel filtration on a Sephadex G-75 superfine column (1.5 × 90 cm) equilibrated with 0.1 M sodium phosphate buffer, pH 7.2, containing 0.15 M sodium chloride. Factor XIIf was a single band of M_r 28,000 on nonreduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Upon reduction, Factor XIIf was a single band of M_r 32,000. Factor XIIf specific activity was 17.9 μmol/min per mg, with S-2302 as the substrate and under conditions described later in this section. The catalytic efficiency of Factor XIIf on S-2302 k_{cat}/K_M was $2.62 \times 10^6\ M^{-1}\ min^{-1}$. Furthermore, incubation of Factor XIIf with purified plasma prekallikrein (26) at a 1:100 enzyme/substrate molar ratio for 15 h at 23°C resulted in the complete activation of prekallikrein to plasma kallikrein, as assessed by SDS-PAGE and kinetic analysis (26). Factor XIIf was radioiodinated with the Bolton-Hunter reagent (27). Its specific radioactivity was 0.3 mCi/mg. Radioactivity was measured with gamma counter (1260 Multigamma; LKB Instruments, Inc., Gaithersburg, MD).

C$\bar{1}$-inhibitor was purified as described by Reboul et al. (28). The resultant preparation, M_r 105,000 on reduced SDS-PAGE, was completely active, as assessed by its reactivity with purified plasma kallikrein (24).

Antithrombin III was purchased from Kabivitrum AB (Molndal, Sweden). It was a single band of M_r 60,000 on reduced SDS-PAGE. The concentration of active material was established by measuring its reaction with plasma kallikrein (29).

α_2-Antiplasmin was prepared using a modification (30) of the procedure described by Wiman (31). The concentration of purified α_2-antiplasmin, a single band of M_r 65,000 on nonreduced SDS-PAGE, was established by titration against plasmin that had been active-site titrated with *p*-NPGB (32).

α_1-Antitrypsin was purified as described (33) and was a gift of Dr. H. L. James. 1 mg of this preparation completely inhibited 0.44 mg of trypsin that had been active-site titrated with *p*-NPGB (32). α_1-Antitrypsin was a single band of M_r 54,000 on reduced SDS-PAGE.

α_2-Macroglobulin was prepared as reported by Sottrup-Jensen et al. (34). Since the resulting preparation exhibited some amidolytic activity on S-2302, it was treated with DFP (10 mM) and then extensively dialyzed. α_2-Macroglobulin was predominantly a single band of M_r 185,000 on reduced SDS-PAGE. It was 85% active, as assessed by its ability to protect active site-titrated trypsin from inactivation by soybean trypsin inhibitor.

Corn trypsin inhibitor, prepared as described (35), was a gift of Dr. E. P. Kirby. This preparation was a single band of M_r 18,000 on reduced SDS-PAGE.

Kinetic studies. Factor XIIf was incubated with various reagents in freshly silicone-coated glass vessels at 23°C. Factor XIIf activity was measured by its amidolytic activity on the chromogenic substrate S-2302. A 0.6-mM solution of the substrate was prepared in 85 mM sodium phosphate buffer, pH 7.6, containing 127 mM NaCl. 10 μl of the solution to be tested was added to 330 μl of substrate at 37°C, and the absorbance change at 405 nm was continuously recorded with a 210 double beam spectrophotometer (Cary Instruments, Varian Associates, Instrument Division, Palo Alto, CA).

Electrophoretic studies and autoradiography. SDS-PAGE was performed as described by Laemmli (36), using vertical slab gels (12 × 16 × 0.15 cm) and a Protean double slab electrophoresis cell (Bio-Rad Laboratories, Richmond, CA). The concentration of acrylamide in the stacking gel was 3%, whereas it was 8.5 or 10% in the separating gel. Electrophoresis was performed at 20–40 mA/gel for 3–4 h. For autoradiography, the gels were exposed at −70°C for 2.5–7 d to Typox RP-L-Film NIF films (Typon AG, Burgdorf, Switzerland) using intensifying screens.

Results

Inactivation of Factor XIIf by purified plasma protease inhibitors: kinetic studies. The kinetics of inactivation of Factor XIIf amidolytic activity by various concentrations of C$\bar{1}$-inhibitor, antithrombin III, and α_2-antiplasmin are illustrated in Figs. 1–3. The inactivation of Factor XIIf followed pseudo-first-order kinetics when these inhibitors were in a 3.5- to 150-fold molar excess. Pseudo-first-order rate constants, k', were obtained by dividing ln 2 by the half-times of enzyme activity. C$\bar{1}$-inhibitor was a more efficient inhibitor of Factor XIIf than was antithrombin III or α_2-antiplasmin. For example, 50% of Factor XIIf amidolytic activity was inactivated by C$\bar{1}$-inhibitor (2.8 μM) in 1.5 min (Fig. 1 *f*), whereas the same proportion of enzyme was inactivated in 18.8 min when antithrombin III was 11 μM (Fig. 2 *b*) and in 10.8 min when α_2-antiplasmin was 10 μM (Fig. 3 *d*). In additional experiments, Factor XIIf was incubated with α_1-antitrypsin at final concentrations that ranged from 27.5 to 55 μM. No reduction in Factor XIIf amidolytic activity was seen after a 30-min incubation at 23°C of Factor XIIf and α_1-antitrypsin (not illustrated). We then investigated the interaction of Factor XIIf with α_2-macroglobulin. 20 μl of Factor XIIf (1.8 μg) was incubated with 20 μl of α_2-macroglobulin (41 μg) or with 20 μl of buffer. After a 10-min incubation at 23°C, these mixtures were assayed for Factor XIIf amidolytic activity. Identical activities were observed, whether Factor XIIf had been incubated with α_2-macroglobulin or with buffer, indicating that Factor XIIf had not reacted with α_2-macroglobulin or that α_2-macroglobulin-bound Factor XIIf had the same amidolytic activity as free Factor XIIf. Since preincubation with α_2-macroglobulin protected trypsin from inactivation by corn trypsin inhibitor, Factor XIIf was then incubated with α_2-macroglobulin under the conditions described above. After a 10-min incubation, the Factor XIIf-α_2-macroglobulin mixture was supplemented either with 35 μl of corn trypsin inhibitor (0.9 mg/ml) or with 35 μl of buffer. 1 min later, Factor XIIf amidolytic activity was assayed. The rate of amidolysis measured after the addition of corn trypsin inhibitor was <0.5% of the rate observed when buffer was added to the Factor XIIf-α_2-macroglobulin mixture. Thus, preincubation of Factor XIIf with α_2-macroglobulin did not prevent Factor XIIf from being inactivated by corn trypsin inhibitor, indicating that no detectable reaction had occurred between Factor XIIf and α_2-macroglobulin.

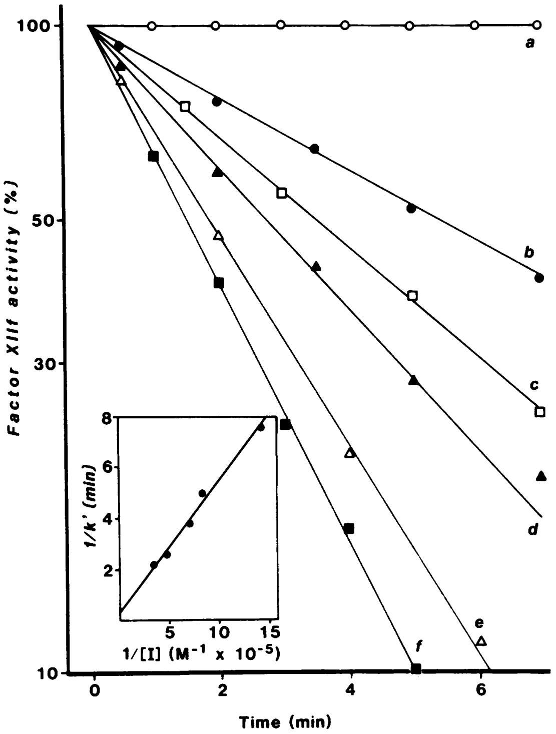

Figure 1. Kinetics of inactivation of Factor XIIf amidolytic activity by C$\bar{1}$-inhibitor. Factor XIIf (final concentration 0.2 μM) was incubated with various concentrations of C$\bar{1}$-inhibitor and then assayed at various times for residual amidolytic activity. C$\bar{1}$-inhibitor final concentrations were: (*a*) 0; (*b*) 0.7; (*c*) 1.2; (*d*) 1.4; (*e*) 2.1; and (*f*) 2.8 μM. The inset shows a double-reciprocal plot of the pseudo-first-order rate constant and the concentration of C$\bar{1}$-inhibitor ([I]). The line drawn is a least-squares fit of the experimental points (r = 0.99). The equation of the line is $y = 0.54x + 0.25$.

The kinetic constants for the inactivation of Factor XIIf by C$\bar{1}$-inhibitor, antithrombin III, and α_2-antiplasmin were derived from double-reciprocal plots of the pseudo-first-order rate constant k' vs the inhibitor concentrations (Figs. 1–3; insets) and are listed in Table I. The second-order rate constants k'' for the reaction of Factor XIIf with these plasma protease inhibitors revealed that the reaction of Factor XIIf with C$\bar{1}$-inhibitor was, respectively, 58 and 20 times faster than the reactions between Factor XIIf and antithrombin III or α_2-antiplasmin (Table I).

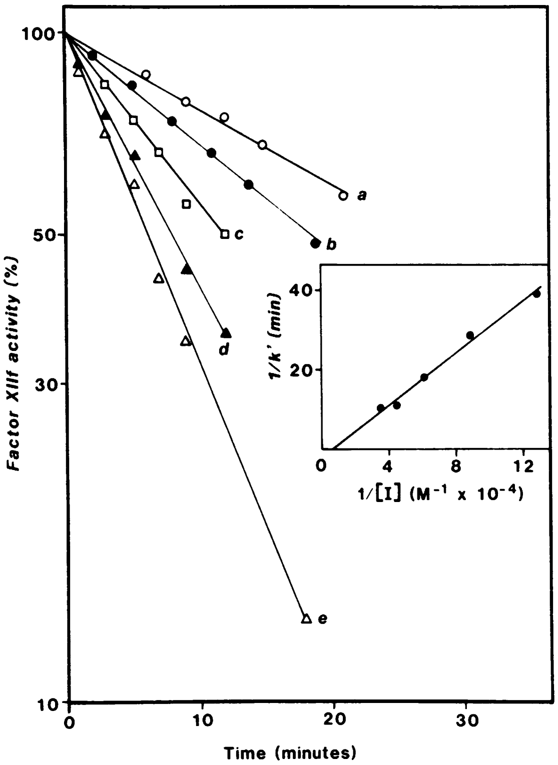

Figure 2. Kinetics of inactivation of Factor XIIf amidolytic activity by antithrombin III. Factor XIIf (final concentration 0.25 μM) was incubated with various concentrations of antithrombin III and then assayed at various times for residual amidolytic activity. Antithrombin III final concentrations were: (*a*) 7.7 μM; (*b*) 11 μM; (*c*) 16.5 μM; (*d*) 22 μM; and (*e*) 27.5 μM. The inset shows a double-reciprocal plot of the pseudo-first-order rate constant and the concentration of antithrombin III ([I]). The line drawn is a least-squares fit of the experimental points ($r = 0.99$). The equation of the line is $y = 3.17x - 2.58$.

Furthermore, the pseudo-first-order rate constants calculated at normal plasma concentration of inhibitors suggested that in normal plasma, C$\bar{1}$-inhibitor would account for 93% of Factor XIIf inhibition, while antithrombin III and α_2-antiplasmin would account for 4 and 3%, respectively (Table I).

Inactivation of Factor XIIf by various plasmas: kinetic studies. The addition of Factor XIIf to plasma containing prekallikrein has been shown to result in the activation of prekallikrein to plasma kallikrein, i.e., in the formation of a species that exhibits amidolytic activity on S-2302. Thus, to evaluate the role of C$\bar{1}$-inhibitor and other plasma protease inhibitors in the inactivation of Factor XIIf in the plasma milieu, we studied the kinetics of inactivation of Factor XIIf amidolytic activity by prekallikrein-deficient plasma. The inactivation of Factor XIIf in a 1:3.5 dilution of the plasma followed pseudo-first-order kinetics. The rate constant for the inactivation of Factor XIIf in prekallikrein-deficient plasma was 14.4×10^{-2} min^{-1} (Fig. 4, curve *a*). This value was reduced to 1.8×10^{-2} min^{-1} in plasma deficient in both prekallikrein and C$\bar{1}$-inhibitor (Fig. 4, curve *b*). These kinetic experiments indicate that C$\bar{1}$-inhibitor is the predominant inhibitor of Factor XIIf in prekallikrein-deficient plasma.

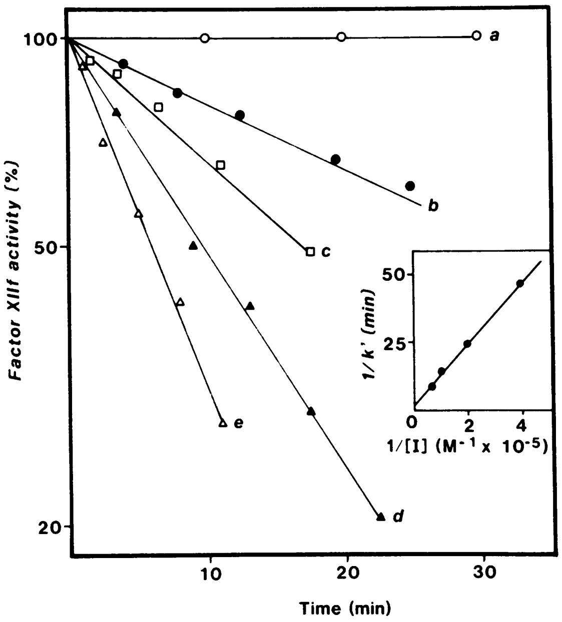

Figure 3. Kinetics of inactivation of Factor XIIf amidolytic activity by α_2-antiplasmin. Factor XIIf (final concentration 0.1 μM) was incubated with various concentrations of α_2-antiplasmin and then assayed at various times for residual amidolytic activity. α_2-Antiplasmin final concentrations were: (*a*) 0; (*b*) 2.5 μM; (*c*) 5.0 μM; (*d*) 10 μM; and (*e*) 15 μM. The inset shows a double-reciprocal plot of the pseudo-first-order rate constant and the concentration of α_2-antiplasmin ([I]). The line drawn is a least-squares fit of the experimental points ($r = 0.99$). The equation of the line is $y = 11.04x + 2.2$.

Table I. Kinetic Constants for the Inactivation of Factor XIIf by C$\bar{1}$-Inhibitor, Antithrombin III, and α_2-Antiplasmin

Inhibitor	Bimolecular reaction rate constant*	Normal plasma concentration‡	Pseudo-first-order inactivation rate constant at normal plasma* concentration
	M^{-1} min^{-1} × 10^{-4}	*μM*	*min^{-1} × 10^{2}*
C$\bar{1}$-inhibitor	18.5	2.2	37.0
Antithrombin III	0.32	4.7	1.50
α_2-Antiplasmin	0.91	1.1	0.97

* Bimolecular reaction rate constants $k'' = k_{+2}/K_i$ and pseudo-first-order inactivation rate constants at normal plasma concentration were calculated as previously described (29, 39). K_i, inhibitor constant.

‡ From references 20 and 37.

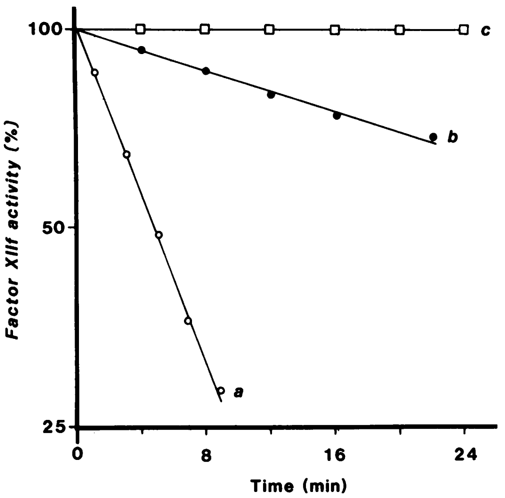

Figure 4. Kinetics of inactivation of Factor XIIf amidolytic activity by plasma. Factor XIIf (final concentration 0.1 μM) was incubated with a 1:3.5 dilution of plasma deficient in prekallikrein (*a*) or plasma deficient in both C$\bar{1}$-inhibitor and prekallikrein (*b*), as well as with buffer (*c*), and then assayed at various times for residual amidolytic activity.

Inactivation of ^{125}I-Factor XIIf by purified plasma protease inhibitors and by various plasmas: SDS-PAGE studies. To confirm the preponderant importance of C$\bar{1}$-inhibitor as a Factor XIIf inhibitor in plasma, we incubated ^{125}I-Factor XIIf in various plasmas and analyzed the resulting mixtures by SDS-PAGE and autoradiography for the appearance of ^{125}I-Factor XIIf-inhibitor complexes. To facilitate the analysis of these studies, ^{125}I-Factor XIIf was first incubated with purified plasma protease inhibitors. The incubation of ^{125}I-Factor XIIf (M_r 28,000; Fig. 5 *a*) with antithrombin III (Fig. 5 *b*), α_2-antiplasmin (Fig. 5 *c*), and C$\bar{1}$-inhibitor (Fig. 5 *e*) resulted in the formation of complexes stable in SDS with apparent M_r of 87,000, 98,000, and 145,000, respectively. These complexes contained approximately one half of the radioactivity, while the other half remained at an M_r of 28,000. This latter fraction was constant in all SDS-PAGE and seemed to represent inactive ^{125}I-Factor XIIf. No complexes that involved ^{125}I-Factor XIIf were formed, owing to the incubation of the radiolabeled enzyme with α_1-antitrypsin (Fig. 5 *d*) and α_2-macroglobulin (Fig. 5 *f*); this is consistent with the kinetic results. Analysis of the mixture formed by the incubation of ^{125}I-Factor XIIf with normal plasma indicated that the active label was associated with three bands with M_r of 145,000, 98,000, and 87,000 (Fig. 6 *g*). These bands were identified as complexes involving Factor XIIf and C$\bar{1}$-inhibitor, antithrombin III, or α_2-antiplasmin, since their migration patterns were identical to those exhibited by these three purified complexes, which were used as internal standards (Fig. 6, *b–d*). Quantitative analysis of four experiments with normal plasma indicated that 74±11% (mean±1 SD) of the active enzyme was forming a complex with C$\bar{1}$-inhibitor in this milieu, while 26±11% of the label was found in the form of complexes that involved Factor XIIf and both α_2-antiplasmin and antithrombin III. ^{125}I-Factor XIIf was also incubated with the plasma from an individual with hereditary angioedema (Fig. 6 *h*). In this plasma, the fraction of active label associated with the band of M_r 145,000 was 42±12% (n = 4), while the fraction associated with the bands of M_r 98,000 and 87,000 was 58±12% (n = 4). When the radiolabeled enzyme was added to hereditary angioedema plasma that had been pretreated with antiserum to C$\bar{1}$-inhibitor, all the active label was found within the bands of M_r 98,000 and 87,000 (Fig. 6 *i*). ^{125}I-Factor was then incubated with plasma deficient in α_2-antiplasmin (Fig. 6 *j*) or in antithrombin III (Fig. 6 *k*). In both plasmas, the active label was predominantly associated with the band of M_r 145,000 (Fig. 6, *j* and *k*) but a small fraction of ^{125}I-Factor XIIf migrated as a species of M_r 87,000 in α_2-antiplasmin-deficient plasma (Fig. 6 *j*) and as a species of M_r 98,000 in antithrombin III-deficient plasma (Fig. 6 *k*). When ^{125}I-Factor XIIf was incubated with plasma deficient in both α_2-antiplasmin and antithrombin III, the active label was entirely associated with the band of M_r 145,000 (Fig. 6, *l*). Finally, the requirement for an active enzyme species to observe labeled complexes with M_r > 28,000 was demonstrated by an experiment in which DFP-treated ^{125}I-Factor XIIf was incubated with normal plasma.

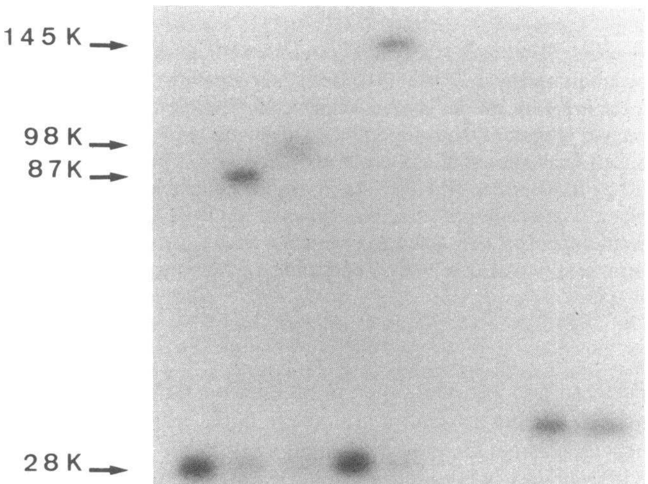

Figure 5. Autoradiogram of SDS-PAGE (10%) analysis of mixtures resulting from the incubation of ^{125}I-Factor XIIf with purified plasma protease inhibitors. Prior to electrophoresis, ^{125}I-Factor XIIF (5 ng) was incubated for 2 h at 37°C in a final volume of 35 μl with either buffer (*a* and *g*); 35 μg antithrombin III (*b*); 10 μg α_2-antiplasmin (*c*); 44 μg α_1-antitrypsin (*d*); 12 μg C$\bar{1}$-inhibitor (*e*); or 25 μg α_2-macroglobulin (*f*). Lanes *a–e* were run under nonreducing conditions; lanes *f–g* were run under reducing conditions.

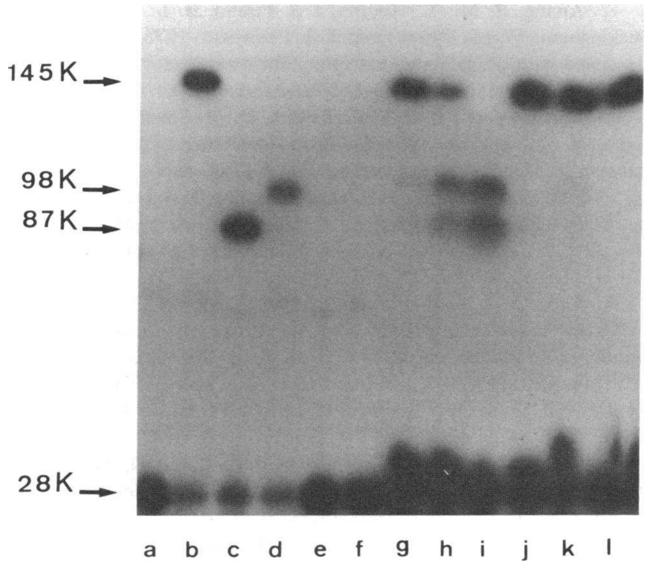

Figure 6. Autoradiogram of SDS-PAGE (8.5%) analysis of mixtures resulting from the incubation of ^{125}I-Factor XIIf with purified plasma protease inhibitors or various plasmas. Before electrophoresis, ^{125}I-Factor XIIf was incubated for 2 h at 37°C with the various reagents. Lanes *a–f:* ^{125}I-Factor XIIf (5 ng) and buffer (*a*); 12 μg C$\bar{1}$-inhibitor (*b*); 35 μg antithrombin III (*c*); 10 μg α_2-antiplasmin (*d*); 44 μg α_1-antitrypsin (*e*); and 25 μg α_2-macroglobulin (*f*). Lanes *g–l:* ^{125}I-Factor XIIf (10 ng in 40 μl) was incubated with 20 μl of either normal plasma (*g*); hereditary angioedema plasma (*h*); hereditary angioedema plasma pretreated with an antiserum to C$\bar{1}$-inhibitor (*i*); α_2-antiplasmin-depleted plasma (*j*); antithrombin III-depleted plasma (*k*); or plasma depleted in both α_2-antiplasmin and antithrombin III (*l*). Nonreducing conditions were employed. For quantitative analysis, each lane of the gel was sliced into six sections, 13 × 8, 20 × 8, 30 × 8, 34 × 8, 17 × 8, and 11 × 8 mm, respectively. Each section was then counted for radioactivity

In this latter experiment, all of the label was found in a single band of M_r 28,000 (not illustrated).

Discussion

This study indicates that C$\bar{1}$-inhibitor is the major inhibitor of Factor XIIf in normal human plasma. This conclusion is supported by (*a*) the analysis of the kinetics of Factor XIIf inactivation in purified systems and in prekallikrein-deficient plasma, and (*b*) the quantitation by SDS-PAGE of the Factor XIIf-inhibitor complexes formed in various plasma as the result of the inactivation of purified radiolabeled enzyme.

To interpret the behavior of Factor XIIf in plasma, we initially investigated the interaction of Factor XIIf with purified plasma protease inhibitors. The second-order rate constant for the reaction of Factor XIIf and C$\bar{1}$-inhibitor was 18.5 × 10^4 M^{-1} min^{-1}, compared with 0.91 and 0.32 × 10^4 M^{-1} min^{-1} for the reactions involving Factor XIIf and α_2-antiplasmin or antithrombin III (Table I). No reaction was detected between the enzyme and plasma concentrations of α_1-antitrypsin or α_2-macroglobulin. Thus, on a molar basis, C$\bar{1}$-inhibitor was the most efficient plasma inhibitor of Factor XIIf. Furthermore, the pseudo-first-order rate constants, determined at a normal plasma inhibitor concentration, indicated that C$\bar{1}$-inhibitor should account for >90% of Factor XIIf inactivation in normal plasma.

The interaction between plasma proteolytic enzymes including Factor XIIf and plasma protease inhibitors results in the formation of enzyme-inhibitor complexes (16, 19, 37, 38). SDS-PAGE analysis of the mixtures resulting from the inactivation of ^{125}I-Factor XIIf (M_r 28,000) by purified C$\bar{1}$-inhibitor (M_r 105,000), α_2-antiplasmin (M_r 67,000), and antithrombin III (M_r 62,000) demonstrated that radiolabeled complexes, with M_r of 145,000, 98,000, and 87,000, respectively, were generated during Factor XIIf inactivation (Fig. 5). The M_r of these complexes are in good agreement with the sum of the M_r of the parent molecules, thereby indicating a 1:1 stoichiometry for the reaction between Factor XIIf and the three inhibitors. No labeled complex was formed as a consequence of the incubation of ^{125}I-Factor XIIf with α_1-antitrypsin and α_2-macroglobulin (Fig. 5). These latter observations strengthened our kinetic results as well as those of an earlier report (18), which indicated that Factor XIIf was not inactivated by α_1-antitrypsin and α_2-macroglobulin.

Kinetic studies revealed that the rate constant for Factor XIIf inactivation in prekallikrein-deficient plasma was 14.4 × 10^{-2} min^{-1}. This constant was in excellent agreement with the expected rate constant (15.5 × 10^{-2} min^{-1}) calculated using kinetic data derived from the study on the inactivation of Factor XIIf by purified C$\bar{1}$-inhibitor, antithrombin III, and α_2-antiplasmin (Table I). In addition, the dominant role of C$\bar{1}$-inhibitor in inactivating Factor XIIf in the plasma milieu was demonstrated by the observation that the rate constant for Factor XIIf inactivation by plasma deficient in both C$\bar{1}$-inhibitor and prekallikrein was reduced to 13% of the rate constant observed when Factor XIIf was inactivated by prekallikrein-deficient plasma (Fig. 4).

The preponderant role of C$\bar{1}$-inhibitor in the inactivation of Factor XIIf in normal plasma was further confirmed by SDS-PAGE analysis and autoradiography of mixtures where ^{125}I-Factor XIIf had been incubated with various plasmas (Fig. 6). When the radiolabeled enzyme was incubated with normal plasma, it was predominantly inactivated by C$\bar{1}$-inhibitor (74%). The difference from the kinetic value of >90% probably derives from the 2-h incubation period used, during which the slower inhibitors would have a chance for maximum inactivation. Similar observations were made with analysis of complexes formed between protease inhibitors and kallikrein (39). Moreover, C$\bar{1}$-inhibitor was still inactivating 42% of the active ^{125}I-Factor XIIf in hereditary angioedema plasma, which contained 32% of the functional C$\bar{1}$-inhibitor of normal plasma. As previously suggested by studies in purified systems (Table I), antithrombin III and α_2-antiplasmin had a minor role in the inactivation of ^{125}I-Factor XIIf in normal plasma (26%), while they became more important in hereditary angioedema plasma (58%).

C$\bar{1}$-inhibitor is known to be the major plasma inhibitor of the proteolytic enzymes derived from the first component of complement (40) and of plasma kallikrein (21, 39), and we now report that C$\bar{1}$-inhibitor is also the predominant plasma inhibitor of Factor XIIf. Patients who lack C$\bar{1}$-inhibitor suffer from hereditary angioedema (41) and present attacks of mucocutaneous swellings and abdominal pain that are associated with unregulated activation of the classical pathway of the complement system (42) and plasma prekallikrein (43). Since both the first component of complement and plasma prekallikrein can be activated by Factor XIIf, we have suggested that the formation of Factor XIIf could be a central biochemical event for inducing angioedema attacks (43). This suggestion is strengthened by the results of the present report, which demonstrate that C$\bar{1}$-inhibitor, the missing protein in patients with hereditary angioedema, is also the major plasma inhibitor of Factor XIIf.

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