THE FIBRINOLYTIC PATHWAY OF HUMAN PLASMA

Isolation and Characterization of the Plasminogen Proactivator*

By ALLEN P. KAPLAN‡ and K. FRANK AUSTEN

(From the Departments of Medicine, Harvard Medical School and Robert B. Brigham Hospital, Boston, Massachusetts 02120)

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The activation of Hageman factor is required for the initiation of three biologically active protein sequences in human plasma. The coagulation sequence proceeds by the action of activated Hageman factor upon precursor plasma thromboplastin antecedent (pre-PTA)[1] (1) and the kinin-generating pathway by the action of activated Hageman factor or fragments derived from activated Hageman factor upon prekallikrein (2, 3). A third substrate of activated Hageman factor or Hageman factor fragments, designated the plasminogen proactivator, has been separated from pre-PTA and from prekallikrein and isolated free of detectable contaminants. After activation by interaction with activated Hageman factor or the Hageman factor fragments, the active enzyme converts plasminogen to plasmin.

Materials and Methods

Bradykinin triacetate (Sandoz Pharmaceuticals Ltd., Basel, Switzerland, or New England Nuclear Corp., Boston, Mass.) was used as the standard for native bradykinin. Antisera to IgG, IgA, IgM, transferrin, albumin, whole human serum, and β_2-glycoprotein I (Behring Diagnostics, Inc., Woodbury, N.Y.); hexadimethrine bromide (polybrene) and diisopropylphosphofluoridate (DFP) (Aldrich Chemical Co., Inc., Milwaukee, Wis.); ϵ-aminocaproic acid (EACA) (Sigma Chemical Co., St. Louis, Mo.); enzodiffusion fibrin plates (Hyland Division, Travenol Laboratories, Inc., Costa Mesa, Calif.); tosyl-L-lysine chloromethyl ketone (TLCK) (Cyclo Chemical Division of Travenol Laboratory, Los Angeles, Calif.); and ampholine carrier ampholytes (LKB, Rockville, Md.) were obtained as indicated. Plasma thromboplastin antecedent (PTA)-deficient plasma and plasma thromboplastin component-deficient plasma

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‡ Formerly, National Institutes of Health Special Research Fellow (2 FO3 AI 42810); present address: Section Head, Allergic Diseases, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

[1] *Abbreviations used in this paper*: CM, carboxymethyl; EACA, ϵ-aminocaproic acid; pre-PTA, precursor plasma thromboplastin antecedent; PTA, plasma thromboplastin antecedent; PTT, partial thromboplastin time; QAE, quaternary aminoethyl; SE, sulfoethyl; TLCK, tosyl-L-lysine chloromethyl ketone.

were obtained from Sera-Tec Biologicals, New Brunswick, N.J. Purified plasminogen was a gift of Dr. E. C. DeRenzo (Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y.).

Serum was processed for the isolation of activated Hageman factor or the Hageman factor fragments, while plasma (4) was utilized as a source of the proenzyme substrates prekallikrein, pre-PTA, and the plasminogen proactivator. Quaternary aminoethyl (QAE) Sephadex (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) and sulfoethyl (SE) Sephadex chromatography were performed utilizing equilibrating buffers containing 0.0035 M PO_4 (0.8 mmho), pH 8.0 and pH 6.0, respectively (4, 5). Sephadex G-100 or G-150 gel filtration (2, 4), disc gel electrophoresis (3), and isoelectric focusing in polyacrylamide gels (4, 5) were performed as previously described.

Preparation of an Anti-IgG Immunoadsorbent. –Human IgG was isolated from plasma so as to be free of detectable prekallikrein, precursor PTA, and the plasminogen proactivator. The effluent obtained from QAE Sephadex chromatography was chromatographed on SE Sephadex, and the 0.08–0.10 M NaCl eluate was then concentrated and chromatographed on Sephadex G-150. The ascending limb of the IgG peak was concentrated to 1 mg/ml, emulsified in complete Freund's adjuvant, and injected into the four footpads of rabbits at a total dose of 1 mg of IgG. 2 wk later, the animals were injected subcutaneously with 1 mg of IgG in incomplete Freund's adjuvant and after another 2 wk were bled from the central artery of the ear. The antiserum diluted $\frac{1}{64}$ gave a visible precipitin band when diffused in Ouchterlony plates against 0.5 mg/ml of IgG immunogen, and the undiluted antiserum gave only a single precipitin arc upon immunoelectrophoresis against whole human serum.

40 ml of the rabbit antiserum were dialyzed against 0.0035 M PO_4 buffer, pH 8.0, and applied to a QAE Sephadex column equilibrated with the same buffer. An initial effluent was collected by applying 400 ml of starting buffer and the column then batch eluted with 0.0035 M PO_4 buffer, pH 8.0, containing 0.035 M NaCl. These two fractions were pooled, and after concentration to the starting volume, exhibited the same anti-IgG titer as the whole rabbit serum. The anti-IgG was coupled to cyanogen bromide-activated Sephadex G-150 (6); coupling was performed between pH 7.5 and 8.0 by addition of 1 M HCl (7). 20 ml of immunoadsorbent were poured into columns and washed with 2 liters of 0.0035 M PO_4 buffer, pH 8.0, containing 0.15 M NaCl. 2 ml of the samples to be adsorbed were applied to the column, the immunoadsorbent was then washed with the equilibrating buffer, and the first 30 ml of effluent concentrated to 2.0 ml.

Preparation and Assay of Components of the Fibrinolytic System. –Activated Hageman factor was prepared by sequential fractionation of plasma on QAE Sephadex, Sephadex G-100, and carboxymethyl (CM)-cellulose, followed by elution from disc gels after electrophoresis at pH 9.3 as previously described (8). Activation of Hageman factor, defined by its ability to correct the coagulation defect of Hageman factor-deficient plasma in the absence of kaolin occurred during the CM-cellulose chromatography. The prealbumin Hageman factor fragments were prepared after partial isolation of unactivated Hageman factor as follows: Plasma dialyzed against 0.0035 M PO_4 buffer in 0.06 M NaCl at pH 8.0 was applied to QAE Sephadex equilibrated with 0.0035 M PO_4 buffer, pH 8.0. The column was batch eluted with the dialysis buffer and the mixture containing unactivated Hageman factor, prekallikrein, pre-PTA, the plasminogen proactivator, and plasminogen was concentrated to the starting volume, activated by stirring in a glass vessel for 24 hr at 4°C, dialyzed against 0.0035 M PO_4 buffer, pH 8.0, and rechromatographed on QAE Sephadex. The Hageman factor fragments eluting between 0.23 and 0.28 M NaCl were purified free of other contaminating proteins by Sephadex G-100 gel filtration, SE Sephadex chromatography, and elution from disc gels as previously described (2). The peak activity of each disc gel eluate was pooled, concentrated, and upon reexamination by disc gel electrophoresis revealed a single prealbumin band. Quantitation on a weight basis was approximated by Folin analysis utilizing albumin as a standard.

Plasminogen was prepared by affinity chromatography of 100 ml of plasma utilizing lysine-Sepharose columns and ϵ-aminocaproic acid elution as described by Deutsch and Mertz (9). After dialysis against 0.0035 M PO_4 buffer in 0.15 M NaCl at pH 8.0, the preparation was fractionated by Sephadex G-100 gel filtration employing the dialysis buffer. The fractions containing plasminogen, as assessed by streptokinase activation and application to fibrin plates, were pooled and concentrated to 10 ml; examination of the plasminogen preparation by disc gel electrophoresis revealed a single broad band identified as plasminogen by functional analysis of an unstained sliced replicate disc gel (Fig. 1). The isoelectric point of the plasminogen based upon functional analysis of the eluates of the acrylamide gel slices ranged from pH 6.3 to 8.6 in agreement with the charge heterogeneity previously reported for purified plasminogen (10). Staining of replicate gels demonstrated multiple bands extending through this pH range.

Plasmin was assayed utilizing Hyland fibrin plates. A standard curve relating ring diameter to plasmin concentration was obtained by activating a reference preparation of purified plasminogen, 250 μg/ml (11), with 140 units of streptokinase for 30 min at 37°C; a linear plot

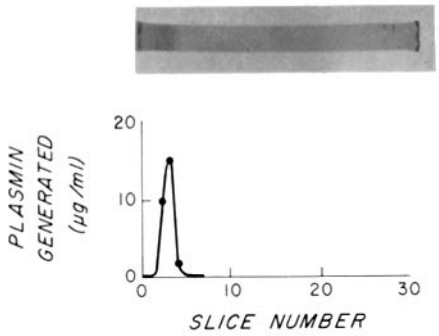

FIG. 1. Alkaline disc gel electrophoresis of purified plasminogen. Beneath is shown the plasmin generated by streptokinase activation of the eluate of each slice of a repilcate, unstained disc gel.

relating log concentration plasmin to ring diameter was obtained between 3 and 250 μg plasmin/ml (Fig. 2). The plasminogen isolated as described above was assayed by incubation with 550 units of streptokinase/mg of plasminogen and had an activity on a weight basis similar to the standard preparation. During isolation procedures, plasminogen was routinely identified by interaction of 100 μl of the various fractions with 5 μl of streptokinase containing 165 units and determining the plasmin generated.

Other substrates of activated Hageman factor, prekallikrein (4), and pre-PTA (4) were assayed functionally as previously described in terms of kinin generation and correction of the partial thromboplastin time of PTA-deficient plasma, respectively. The inhibitor of the activated first component of complement (C$\bar{1}$INH), prepared by a modification of the method described in (12), gave no precipitin band upon immunoelectrophoretic analysis with antiserum to whole human serum, to α_1-antitrypsin, or to α_2-macroglobulin but did precipitate with antiserum to C$\bar{1}$INH.

RESULTS

Purification of the Plasminogen Proactivator

In order to seek the precursor form of a protein involved in plasminogen activation, fractions of human plasma were interacted with the Hageman

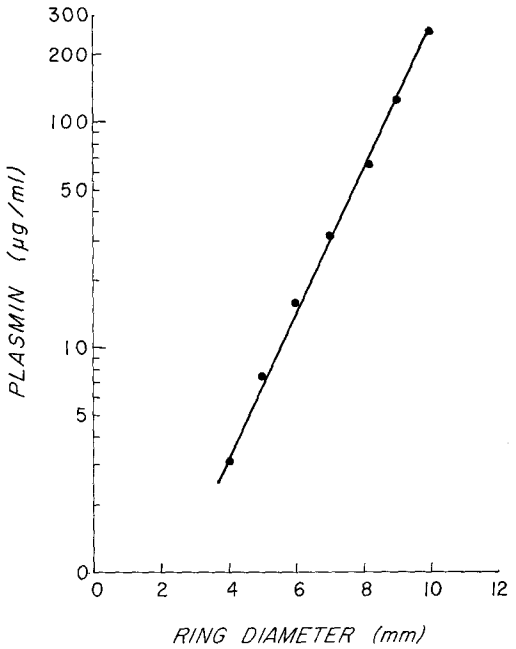

FIG. 2. Plot relating ring diameter in a fibrin plate with the log concentration of plasmin.

factor fragments and then with plasminogen. The effluent obtained from QAE Sephadex chromatography of 100 ml of plasma, upon interaction with the Hageman factor fragments, led to the conversion of plasminogen to plasmin (Fig. 3). The effluent had no plasminogen-converting activity in the absence of the Hageman factor fragments. Neither the effluent nor the eluate contained kallikrein, PTA, activated Hageman factor, Hageman factor fragments, or plasmin, indicating that these enzymes had remained in the precursor state during chromatography. After application of a sodium chloride gradient, unactivated Hageman factor was eluted between 0.04 and 0.06 M NaCl and plasminogen between 0.06 and 0.09 M NaCl; none of the eluate fractions contained material capable of converting plasminogen to plasmin with or without the addition of Hageman factor fragments.

The effluent from the QAE Sephadex chromatogram was pooled, concentrated to 10 ml, and again assayed for its capacity to convert plasminogen to plasmin. The failure of streptokinase alone to induce fibrinolysis in the plate demonstrates that the fibrin plate lacked plasminogen. Incubation of Hageman factor fragments with plasminogen alone or incubation of the QAE Sephadex effluent with plasminogen gave no conversion to plasmin. Incubation of the Hageman factor fragments with the effluent for 10 min at 37°C followed by incubation of the mixture with plasminogen, 200 μg/ml, for 1 hr at 37°C yielded a ring of fibrinolysis equivalent to the generation of 80 μg of plasmin, confirming

the presence of a function designated the plasminogen proactivator in the concentrated effluent (Fig. 4).

The concentrated QAE Sephadex effluent was then chromatographed on SE Sephadex to determine the relationship of the plasminogen-activating activity

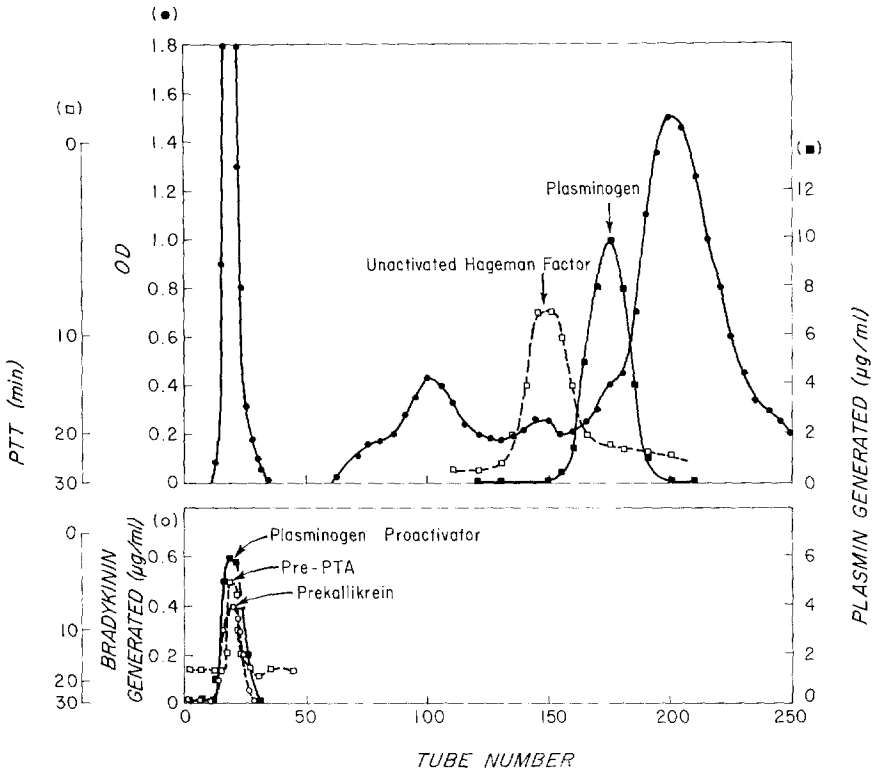

FIG. 3. QAE Sephadex chromatography of plasma with analysis of the eluate (above) and effluent (below).

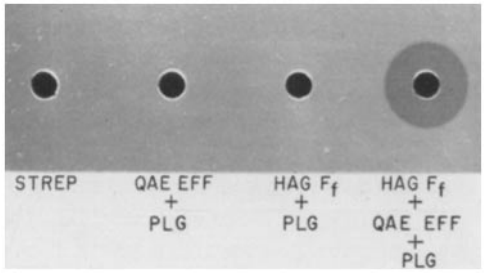

FIG. 4. Fibrin plate assay of the concentrate of the QAE Sephadex effluent shown in Fig. 3. *STREP*, streptokinase; *QAE EFF*, QAE Sephadex effluent; *PLG*, plasminogen; *HAG* F_f, Hageman factor fragments.

to the pre-PTA and prekallikrein also present in this fraction (Fig. 5). After application of a linear sodium chloride gradient, prekallikrein was eluted between 0.12 and 0.14 M NaCl, precursor PTA between 0.16 and 0.19 M NaCl, and the plasminogen proactivator in a region between but overlapping both prekallikrein and pre-PTA. The plasminogen proactivator peak shown in Fig. 5 was divided in half by pooling tube Nos. 90 to 120 and Nos. 121 to 160, so as to obtain one pool contaminated predominantly with prekallikrein and the other with precursor PTA and to a lesser degree with prekallikrein. Each pool was then concentrated to 2 ml and fractionated on Sephadex G-150. The first SE Sephadex pool, as shown in Fig. 6 A, revealed prekallikrein along the descending limb of the IgG peak and the plasminogen proactivator along the descending limb of the prekallikrein peak. The second SE Sephadex pool, shown in Fig. 6 B, revealed pre-PTA along the ascending portion of the IgG peak and a

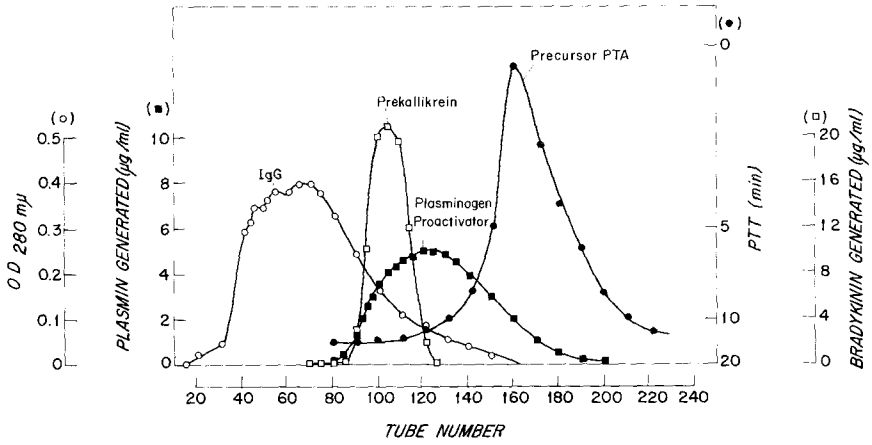

FIG. 5. SE Sephadex chromatography of the QAE Sephadex effluent shown in Fig. 3.

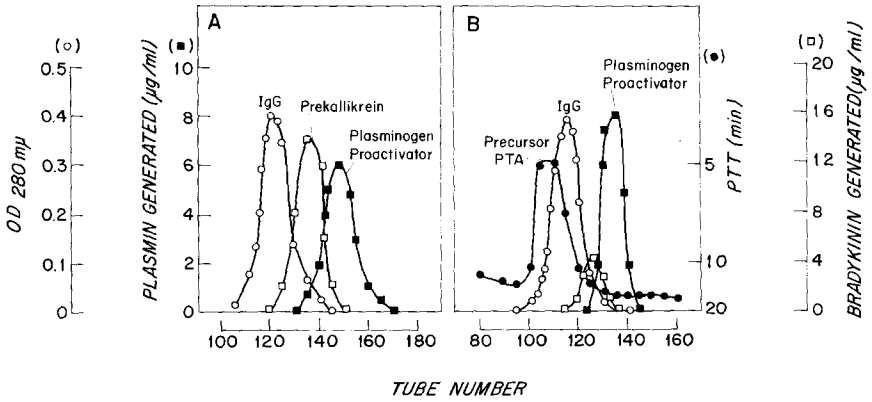

FIG. 6. Sephadex G-150 chromatography of pools from tube Nos. 90 to 120 (*A*) and tube Nos. 121 to 160 (*B*) obtained from SE Sephadex (Fig. 5).

small prekallikrein peak preceding the position of the plsasminogen proactivator. When the plasminogen proactivator peak shown in Fig. 6 B was pooled, concentrated to 2 ml, and rechromatographed on Sephadex G-150, the plasminogen proactivator obtained after concentration to the starting volume was free of prekallikrein and contained trace quantities of IgG as the sole identifiable contaminant. The entire sample was then applied to the anti-IgG immunoadsorbent, the effluent concentrated to 2 ml, and a portion focused in polyacrylamide gels utilizing ampholytes ranging from pH 7 to 10. As shown in Fig. 7, the stained gel had a single band; functional assessment of a replicate

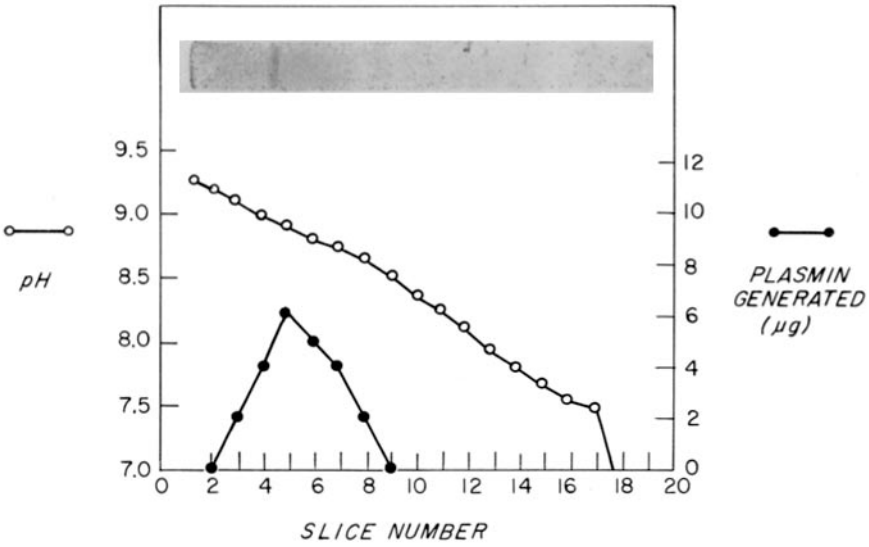

FIG. 7. Isoelectric focusing in polyacrylamide gels of purified plasminogen proactivator utilizing pH 7–10 ampholytes. Beneath is shown the assay for plasminogen proactivator of the eluate of each slice of a replicate, unstained gel.

sliced, unstained gel revealed the plasminogen proactivator peak at pH 8.9, the position of the stained band.

Prekallikrein (Fig. 6 A) and pre-PTA (Fig. 6 B) obtained from the Sephadex G-150 step were isoelectric at pH 8.75 and pH 9.1, respectively, as determined by elution and functional assessment, while plasminogen proactivator was isoelectric at pH 8.9. Fractionation of pre-PTA, prekallikrein, and the plasminogen proactivator obtained at the Sephadex G-150 step in the presence of substances of known molecular weight revealed estimated molecular weights of 175,000 for pre-PTA, 127,000 for prekallikrein, and 100,000 for the plasminogen proactivator (Fig. 8).

Conversion of Plasminogen to Plasmin by the Plasminogen Activator

The plasminogen proactivator peak at the stage of purification illustrated in Fig. 6 B was concentrated to 2 ml, and 100 μl were incubated with 2 μl of

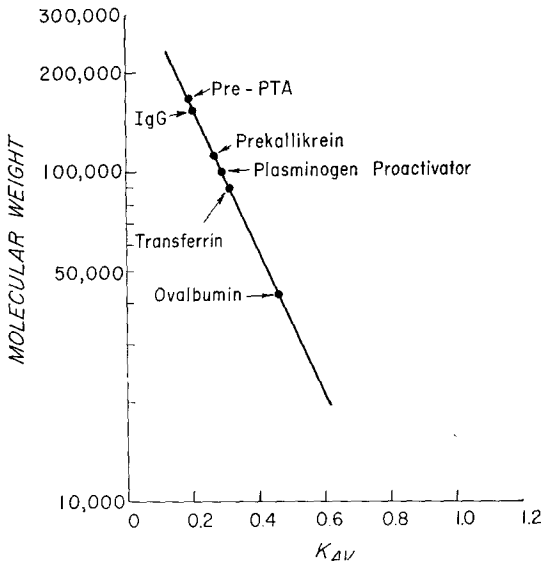

FIG. 8. Molecular weight estimation of three substrates of active Hageman factor by gel filtration on Sephadex G-150. The K_{av} of the marker proteins IgG, transferrin, and albumin are shown on the left.

Hageman factor fragments, 25 μg/ml, for 1 hr at 37°C. 20 μl of this mixture were then incubated with 20 μl of plasminogen, 200 μg/ml, for 1 hr at 37°C, and 46 μg/ml plasmin was generated. Incubation of the plasminogen proactivator with quantities of the Hageman factor fragments up to 2.5 μg for periods ranging from 1 to 6 hr did not reveal further plasminogen activator activity.

The plasminogen activator formed as described above from 100 μl of plasminogen proactivator and 2 μl of Hageman factor fragments was diluted as shown in Fig. 9 and 20 μl of each dilution incubated with 20 μl of plasminogen for 2 hr at 37°C. A straight line was obtained relating dilution of plasminogen activator to plasmin generated. These dilutions of plasminogen activator gave the same yield of plasmin when the plasminogen substrate was diluted 1:4 so that the maximum plasminogen made available by streptokinase activation was 50 μg/ml. Incubation of the plasminogen activator with excess plasminogen for periods up to 3 hr gave minimal further increases in the quantity of plasmin as compared with incubation for 15 min; incubation for a further 9 hr revealed conversion of additional plasminogen to plasmin.

Inhibition of the Plasminogen Activator and Plasmin

Active Site Inhibitors.—DFP, an active site serine inhibitor, and TLCK, an active site histidine inhibitor, were examined for their ability to inhibit the plasminogen activator and plasmin. Plasminogen proactivator obtained from the Sephadex G-150 step (Fig. 6 B) and activated as described above generated

50 μg/ml of plasmin from plasminogen. The plasminogen activator was incubated for 90 min at 37°C with either buffer alone, or sufficient DFP or TLCK to yield a final concentration of 10^{-3} M. Each reaction mixture was then dialyzed overnight against 0.0035 M PO_4 buffer, pH 8.0, containing 0.015 M NaCl. The same preparation of plasminogen proactivator was incubated with the same materials, dialyzed overnight, and then activated with the Hageman factor fragments. 20 μl of each of these six samples were then incubated with 20 μl of plasminogen, 200 μg/ml, for 1 hr at 37°C, and the conversion to plasmin was assessed on fibrin plates. As shown in Fig. 10, incubation of the plasminogen

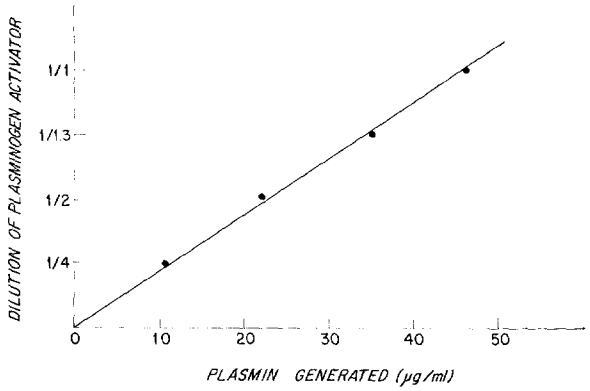

FIG. 9. Graph relating dilution of plasminogen activator to plasmin generated from a constant input of plasminogen.

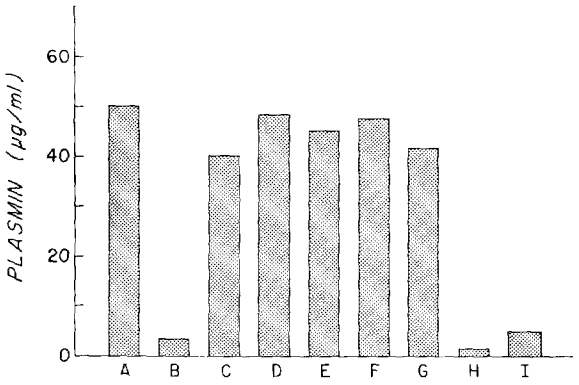

FIG. 10. Inhibition of the plasminogen activator and plasmin by DFP and TLCK. (*A*) Plasminogen activator and plasminogen. (*B*) Plasminogen activator made 10^{-3} M in DFP and plasminogen. (*C*) Plasminogen activator made 10^{-3} M in TLCK and plasminogen. (*D*) Plasminogen proactivator and Hageman factor fragments and plasminogen. (*E*) Plasminogen proactivator made 10^{-3} M in DFP and Hageman factor fragments and plasminogen. (*F*) Plasminogen proactivator made 10^{-3} M in TLCK and Hageman factor fragments and plasminogen. (*G*) Plasmin made 10^{-3} M in DFP. (*H*) Plasmin made 10^{-3} M in TLCK. (*I*) Plasmin made 10^{-2} M in DFP.

activator with DFP (*B*) resulted in 90% inhibition, while incubation with TLCK resulted in 20% inhibition (*C*). The plasminogen proactivator retained over 90% of its ability to convert plasminogen to plasmin (*D*, *E*, *F*). The plasmin resulting from the interaction of the plasminogen activator and plasminogen (*A*) was made 10^{-3} M in DFP or 10^{-3} M in TLCK, dialyzed, and its functional integrity determined. 10^{-3} M DFP resulted in 18% inhibition of plasmin (*G*), whereas 10^{-3} M TLCK yielded 96% inhibition (*H*). Increasing the dose of DFP to 10^{-2} M yielded 90% plasmin inhibition (*I*).

Action of EACA.—EACA, a known inhibitor of plasmin (13) and of plasminogen activation (13, 14), was examined for its effect upon the plasminogen activator. A preparation of plasminogen proactivator obtained from the Sephadex G-150 step (Fig. 6 B) was activated and generated 90 μg/ml of plasmin from plasminogen. This plasminogen activator was incubated with plasminogen for 1 hr at 37°C in the presence of final concentrations of EACA ranging from 0.025 M to 0.2 M. For comparison, the plasminogen activator was incubated with plasminogen for 1 hr at 37°C and the EACA added subsequently to achieve the same final concentrations as above. The incubation with EACA was carried out for 1 hr before assay. The area between the two plots reflects the capacity of EACA to prevent conversion of plasminogen to plasmin by the plasminogen activator beyond any effect on the assay through inhibition of the plasmin generated (Fig. 11).

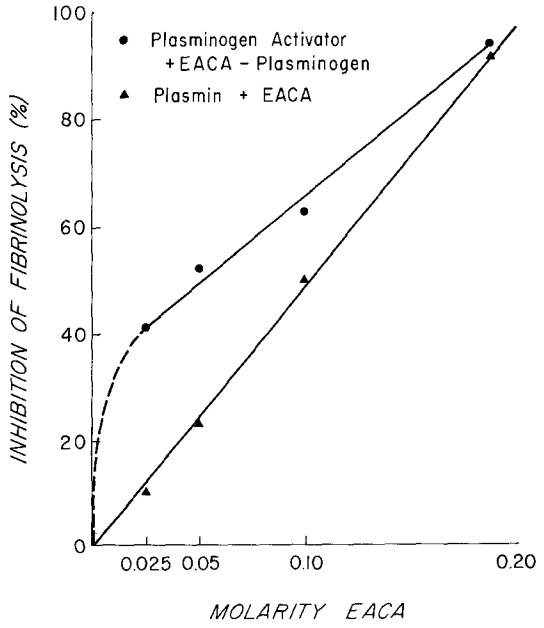

FIG. 11. The effect of EACA at concentrations ranging from 0.025 M to 0.2 M upon plasminogen activation and upon plasmin.

Effect of $C\bar{1}INH$.—1 ml of the concentrated QAE Sephadex effluent (Fig. 3) was activated with 50 μl of Hageman factor fragments, 25 μg/ml, to yield kallikrein and plasminogen activator. 500 μl of the reaction mixture were incubated with either equal volumes of buffer or $C\bar{1}$INH 130,000 units/ml. The mixtures were dialyzed for 4½ hr against 0.0035 M PO_4 buffer, pH 8.0, and each was applied to a 2 × 10 cm column of QAE Sephadex previously equilibrated with the dialysis buffer. 30 ml of effluent were collected, concentrated to 1 ml, and assayed for kallikrein and plasminogen activator. As shown in Table I, 5000 ng of bradykinin/ml were generated from heat-inactivated plasma as a source of kininogen while less than 10 ng of bradykinin was generated when the initial effluent was previously interacted with $C\bar{1}$INH to inhibit kallikrein. In contrast, the effluent generated the same quantity of plasmin from plasminogen (32–33 μg/ml) irrespective of the prior interaction of the plasminogen activator of the initial effluent with the $C\bar{1}$INH. $C\bar{1}$INH was not present in the effluent

TABLE I

Effect of $C\bar{1}INH$ on Kallikrein vs. Plasminogen Activator

	Kallikrein	Plasminogen activator
	ng bradykinin/ml generated	*μg plasmin/ml generated*
QAE effluent and buffer	5000	33
QAE effluent and $C\bar{1}$INH	<10	32

as assessed by hemolytic titrations and thus the $C\bar{1}$INH input could not have influenced the assay of kallikrein.

DISCUSSION

The activation of Hageman factor in plasma is known to result in the development of fibrinolytic activity which is not attributable to the direct action of activated Hageman factor (15–17) or its fragments (Fig. 4) upon plasminogen. The conversion of plasminogen proactivator to plasminogen activator by activated Hageman factor has been recognized as an essential step in the fibrinolytic sequence. The plasminogen proactivator was isolated from normal human plasma by sequential chromatography using QAE Sephadex (Fig. 3), SE Sephadex (Fig. 5), and Sephadex G-150 gel filtration (Fig. 6). The location of the plasminogen proactivator during the chromatographic procedures was recognized using purified Hageman factor fragments for activation and purified plasminogen (Fig. 1) as substrate for plasminogen activator (Fig. 7). These procedures separated plasminogen proactivator from two other proenzyme substrates of activated Hageman factor, prekallikrein and pre-PTA (Figs. 5, 6). The contaminating IgG was then removed by using an immunoadsorbent and the final preparation of plasminogen proactivator gave a single band on disc

gel electrophoresis or isoelectric focusing in polyacrylamide gels which corresponded to the position of the functional material eluted from unstained gels (Fig. 7).

The isoelectric point of the plasminogen proactivator, pH 8.9 (Fig. 7), is intermediate between that of the other two substrates of active Hageman factor, kallikrein, pH 8.75; and pre-PTA, pH 9.1. The plasminogen proactivator has an estimated molecular weight of 100,000 (Fig. 8) by Sephadex G-150 gel filtration, a procedure giving an estimated molecular weight of 127,000 for prekallikrein and 175,000 for pre-PTA. Although the proenzymes of kallikrein and plasminogen activator are similar in size and isoelectric point, a difference in the functional characteristics of their active sites further distinguished these two proteins. Kallikrein is known to be inhibitable by $C\bar{1}INH$ (18, 19) and when present in a mixture with plasminogen activator, kallikrein was completely inhibited by a concentration of $C\bar{1}INH$ which had no effect upon the plasminogen activator (Table I). The active site in plasminogen activator is susceptible to inactivation by DFP (Fig. 10) but not by a comparable concentration of TLCK. The active site in the precursor molecule, the plasminogen proactivator, is protected from DFP inactivation as has been the case for many proesterases having a serine in the active site.

Ogston et al. have described an active enzyme dependent upon prior Hageman factor activation, termed Hageman factor cofactor, which is required for the fibrinolytic pathway (17). The plasminogen activator is similar to the Hageman factor cofactor in its properties during ion exchange chromatography and in the inability of $C\bar{1}INH$ to inhibit its action upon plasminogen. However, the estimated molecular weight of the Hageman factor cofactor (165,000) is greater than that of the plasminogen activator isolated directly from serum, which is comparable in size to the plasminogen proactivator (100,000). The isolation of Hageman factor cofactor following a variety of precipitation procedures could have favored formation of a plasminogen activator-plasmin complex or may have permitted aggregation.

The interaction of the plasminogen activator with plasminogen for a limited time period is characterized by a yield of plasmin proportional to the plasminogen activator concentration (Fig. 9). When there is plasminogen excess during the limited time period, as assessed by streptokinase activation, only a fraction of the available plasminogen is activated for a given input of plasminogen activator. These reaction characteristics are similar to the kinetics of plasminogen activation reported by Colman utilizing a kallikrein source, presumably containing plasminogen activator, to activate plasminogen and casein as the plasmin substrate (20). Such kinetics are consistent with binding of plasminogen activator with plasminogen to form a complex. After prolonged interaction of plasminogen activator and plasminogen, there is a second phase of plasminogen activation. These two steps are reminiscent of the interaction of streptokinase with plasminogen. Initially, a streptokinase-plasminogen complex forms

stoichiometrically, converts to an active streptokinase-plasmin complex (21–23), and this complex or a fragment therefrom (24, 25) activates plasminogen catalytically (22, 24). Takada et al. have described a streptokinase activatable proactivator of plasminogen which is present in human plasma; at very low doses of streptokinase, this factor may be activated to a plasminogen activator with little direct activation of plasminogen by the streptokinase (26). The plasminogen proactivator described herein is not activatable by streptokinase. Further the plasminogen proactivator is not converted to plasminogen activator by human plasmin derived by streptokinase activation or by plasmin developing spontaneously during the affinity chromatographic isolation of plasminogen. Finally, the plasminogen activator, kallikrein, and PTA do not activate the plasminogen proactivator. Intact activated Hageman factor or its fragments represent the only molecules recognized thus far capable of converting the plasminogen proactivator to plasminogen activator.

A summary of the pathways resulting from the activation of three Hageman factor substrates is shown in Fig. 12. Although the Hageman factor prealbumin fragments have been routinely used to activate the plasminogen proactivator in the present studies, intact activated Hageman factor or any of the previously described intermediate sized fragments of activated Hageman factor (3) readily activate the plasminogen proactivator. Intact activated Hageman factor is converted to the fragments by digestion with plasmin (3) thus diverting the reaction sequence from coagulation to kinin generation. The insertion of EACA at the time of Hageman factor activation in the fluid phase has been found to protect the active Hageman factor from fragmentation and to diminish the rate of conversion of prekallikrein to kallikrein (8). The capacity of EACA to bind to plasminogen (9, 27, 28) and inhibit the action of the plasminogen

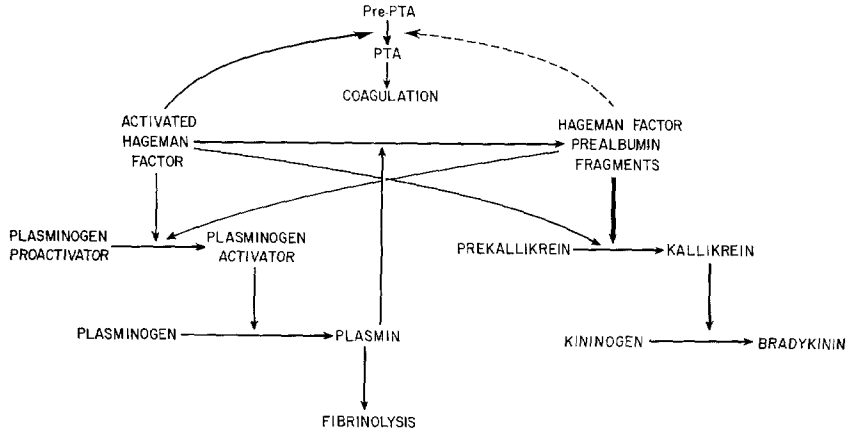

FIG. 12. The interrelationship of the fibrinolytic sequence with the coagulation and kinin-generating systems.

activator upon plasminogen (Fig. 11) would be consistent with such an effect and with the over-all reaction scheme as postulated.

SUMMARY

The conversion of the plasminogen proactivator to plasminogen activator by activated Hageman factor or its fragments has been recognized as an essential step in the conversion of plasminogen to plasmin. The plasminogen proactivator has been completely separated from prekallikrein and pre-PTA, two other proenzyme substrates of activated Hageman factor or its fragments. Plasminogen proactivator, free of any contaminating proteins as assessed by disc gel electrophoresis or isoelectric focusing, revealed a single band with an isoelectric point of 8.9 corresponding in position to the Hageman factor activatable material eluted from replicate unstained gels. After conversion of plasminogen proactivator by Hageman factor fragments to the plasminogen activator, the active site of the plasminogen activator is not inhibited by $C\bar{1}INH$ and is thus readily distinguished from that of kallikrein or PTA. The plasminogen activator is susceptible to inactivation by DFP while the plasminogen proactivator is not, as has been the case for esterases having a serine in the active site. Its interaction with plasminogen is inhibited by ϵ-aminocaproic acid.

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