Isolation and Properties of an Abnormal Hageman Factor (Factor XII) Molecule in a Cross-reacting Material-positive Hageman Trait Plasma

HIDEHIKO SAITO and SALVATORE J. SCIALLA, *Department of Medicine, Case Western Reserve University School of Medicine, University Hospitals of Cleveland, Cleveland, Ohio 44106; Walter Reed Army Medical Center, Washington, D.C. 20012*

ABSTRACT We have previously described two unrelated individuals with homozygous Hageman trait (Factor XII deficiency) whose plasmas contained nonfunctional material immunologically indistinguishable from normal Hageman factor (HF). Abnormal HF from the plasma of one of these subjects has now been purified to homogeneity, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, alkaline disc gel electrophoresis, and immunoelectrophoresis. Purified abnormal HF had no clot-promoting activity, but showed the same specific antigenicity as purified normal HF by an immunoassay. The abnormal HF was of a single chain polypeptide with the same molecular weight (80,000) as normal HF and was positively stained by periodic acid-Schiff reagent. Both normal and abnormal HF had similar amino acid compositions and isoelectric points (pI 6.5 ~ 7.1). When ^{125}I-labeled abnormal HF and ^{131}I-labeled normal HF were mixed with normal plasma and exposed to glass, both HF underwent an identical pattern of cleavage, yielding 52,000- and 30,000-mol wt fragments. Similarly, abnormal HF was fragmented by trypsin in the same way as normal HF, but no prekallikrein-activating activity was generated after cleavage. [^{3}H]Diisopropyl phosphorofluoridate was incorporated into a 29,000-mol wt fragment of the trypsin-cleaved normal HF, but not into that of the trypsin-cleaved abnormal HF. These data suggest that the molecular defect in this abnormal HF resides at or near the active site serine residue in the 30,000-mol wt part of the molecule.

INTRODUCTION

Hageman factor (HF,[1] Factor XII) is a plasma protein that is functionally deficient in plasmas from individuals with Hageman trait. Under certain conditions, HF appears to trigger such surface-mediated plasma reactions as the intrinsic pathway of blood coagulation, contact-activated fibrinolysis, and kinin generations (1).

Although the majority of plasmas of subjects with congenital Hageman factor deficiency also lack immunologically identifiable HF, two unrelated individuals with this disorder have recently been found whose plasmas contained essentially normal amounts of nonfunctional, but immunologically indistinguishable HF (2). Inasmuch as these plasmas contained cross-reacting material (CRM) recognized by the specific antiserum, these cases have been termed CRM^{+} (CRM-positive variant). Elucidation of the nature of the molecular defect in these abnormal HF may provide a unique opportunity to examine the structure-function relationship of this plasma protein.

In the present study, we have isolated an abnormal HF from plasma of an individual with CRM^{+} Hageman trait and have studied some of its properties in

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Address reprint requests to Dr. H. Saito, Department of Medicine, University Hospitals of Cleveland.

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[1] *Abbreviations used in this paper*: BS, barbital-saline buffer, CRM, cross reacting material; DFP, diisopropyl phosphorofluoridate; HF, Hageman factor; LBTI, lima bean trypsin inhibitor; RIA, radioimmunoassay; RT, room temperature; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SP, sulfo-propyl; TLCK, L-choro-tosylamido-amino-heptanone.

comparison with normal HF. This abnormal HF appears to have a defective active site in the light chain of the molecule.

METHODS

Citrated plasma from normal individuals and subjects with the usual form of Hageman trait were prepared as described earlier (2). Plasmas from an individual with CRM^+ Hageman trait (subject 2 in ref. 2) were obtained, after informed consent, by plasmaphoresis, using acid-citrate-dextrose (formula A, U. S. Pharmacopeia) anticoagulant. Normal plasma for the isolation of HF was obtained by collecting blood into 1:6 vol of the same anticoagulant.

DEAE-Sephadex A-50, SP-Sephadex C-50, Sephadex G 150, and CNBr-activated Sepharose 4B were purchased from Pharmacia Fine Chemicals Inc., Piscataway, N. J. Barbital-saline buffer (BS) was 0.025 M sodium barbital in 0.125 M sodium chloride at pH 7.5. Crude immunoglobulin fraction of rabbit anti-HF serum (3) was coupled to CNBr-activated Sepharose 4B according to the manufacturer's instructions.

Hageman factor clot-promoting assays and double-antibody radioimmunoassays (RIA) were performed as described earlier (3). A standard pool of 24 normal male plasmas was prepared by an earlier method (3) and was used as the standard for the measurement of both HF clot-promoting activity and HF antigen (immunoreactive HF). The pooled plasma was arbitrarily said to contain 1.0 U activity/ml and 1.0 U antigen/ml.

A solid-phase RIA for HF was developed to monitor the HF antigen of a CRM^+ Hageman trait plasma during isolation, since a fluid-phase double-antibody RIA (3) takes a few days to complete and is not suitable for rapid determination during the purification steps. The assay mixture consisted of 0.2 ml BS containing 0.05% Tween 20 (Supelco, Inc., Bellefonte, Pa.) and 0.2% bovine serum albumin (Pentex, Kankakee, Ill.), 0.02 ml ^{125}I-HF (usually ~12,000 cpm), 0.05 ml test material, and 0.1 ml Sepharose-bound anti-HF IgG in a 10 × 75-mm polystyrene tube. The tube was shaken for 90 min at room temperature (RT) by a wrist action shaker (Burrell Corp., Pittsburgh, Pa.). Ice-cold BS (1 ml) was then added to the tube and the tube was immediately centrifuged at 1,800 *g* for 10 min at 2°C. The supernate was discarded, and the precipitated Sepharose was washed and counted for radioactivity in a γ-counter (model 1085, Nuclear Chicago, Des Plaines, Ill.). The whole procedure took <3 h. Duplicate determinations were performed for each sample, and serial dilutions of normal pooled plasma were included in each run. A standard curve was prepared by plotting the bound counts per minute against the logarithm of the concentration of HF and was essentially linear between 1.0 and 0.05 U HF/ml.

Isolation of the normal and abnormal HF was carried out essentially by the method of Griffin and Cochrane (4). Purified HF was stored at 4°C in 0.05 M sodium acetate buffer (pH 5.2) containing 0.15 M NaCl and 0.5 mM ethylenediaminetetraacetic acid (EDTA).

Radioiodination of purified normal and abnormal HF with ^{125}I and ^{131}I was performed by a chloramine-T method (5). The ^{131}I-labeled normal HF had 1.3×10^7 cpm/μg HF, and ^{125}I-labeled normal HF had 2.5×10^7 cpm/μg HF. The ^{125}I-labeled abnormal HF had 2.4×10^7 cpm/μg HF. Protein was estimated by the method of Lowry et al. (6).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Weber and Osborn (7), by use of 6% gels. Gels were stained for protein with Coomassie Blue or for carbohydrate with periodic acid-Schiff reagent (8). A low molecular-weight calibration kit (Pharmacia Fine Chemicals) consisting of six polypeptides of known molecular weight was used to estimate molecular weight.

Analytical disc gel electrophoresis was performed according to a published method (9) using 5 × 75-mm gels. Electrophoresis was run at 5 mA per gel for 45 min, and gels were stained with Coomassie Blue. Replicate gels were frozen and sliced into 2-mm segments, and each segment was incubated with 0.4 ml BS containing 1 mg/ml bovine serum albumin overnight at 4°C. The sample was centrifuged and the supernate was assayed for both HF activity and HF antigen.

Isoelectric focusing was carried out by the method of Righetti and Drysdale (10) using 5 × 120-mm gels prepared with 6% acrylamide and 2% pH 3.5–10 Ampholine (LKB, Stockholm, Sweden). Electrophoresis was performed at 200 V for 19 h at RT. The gels were either stained with 0.04% Coomassie Brilliant Blue G-250 in 3.5% perchloric acid (11) or sliced into 5-mm segments for pH determination and for the identification of HF by RIA. Two markers with known isoelectric points (pI), Evans blue (General Diagnostics, Morris Plains, N. J., pI 5.4) and ovalbumin (twice crystallized, Schwarz/Mann, Orangeburg, N. Y., pI 4.7) were run simultaneously for comparison.

Immunodiffusion was performed in a 1% agarose gel in 0.05 M sodium barbital buffer (pH 8.4) for 2 d at RT. Immunoelectrophoresis was carried out in 1% agarose gels in 0.05 M sodium barbital buffer (pH 8.4) at a current of 10 V/cm for 5 h at RT. 0.2 ml of the crude immunoglobulin fraction of rabbit anti-HF serum was then placed in the trough, and precipitin lines were allowed to develop over 48 h at RT.

Amino acid analysis of purified normal and abnormal HF were carried out with a Durrum D 500 Amino Acid Analyser (Durrum Instrument Corp., Palo Alto, Calif.). Normal or abnormal HF (50 μg) was hydrolyzed at 110°C in 6 N HCl for 24 h. Half-cystine was determined as cysteic acid (12) after performic acid oxidation. No value for tryptophan was reported, because it is destroyed by acid hydrolysis. The results reported were the average of duplicate determinations.

Cleavage of ^{131}I-labeled normal HF, ^{125}I-labeled normal HF, and ^{125}I-labeled abnormal HF in the contact activation of plasma was studied by the method of Revak et al. (13). ^{131}I-normal HF (19 ng) and ^{125}I-abnormal HF (14 ng)—2.5 μl of each—were mixed with 20 μl of normal plasma and 175 μl of BS in a polystyrene tube. The final plasma dilution was 1:10 and the radiolabeled proteins that were added did not increase the plasma content of HF by >5%. 20 μl of this mixture was added to 10 × 75-mm glass tubes and shaken at RT for various times. The reactions were terminated by the addition of 50 μl of 4% SDS with or without 4% 2-mercaptoethanol, and the samples were subjected to SDS-PAGE after boiling for 3 min. The zero time sample was obtained by adding the diluted plasma containing radiolabeled HF directly to the SDS solution. Gels were sliced into 2-mm segments, and each slice was counted for ^{131}I and ^{125}I radioactivity with separate windows. ^{125}I radioactivity was corrected for the spillover from the ^{131}I to the ^{125}I window. Greater than 90% of the radioactivity applied to the gel was recovered. Similar experiments were performed by exposing a CRM^+ Hageman trait plasma containing either ^{125}I-abnormal HF alone, ^{125}I-abnormal HF plus nonradioactive normal HF, or ^{125}I-normal HF alone to glass. The results were expresed as the percentage of the radioactivity of each slice relative to the total radioactivity recovered.

Cleavage of ^{131}I-labeled normal HF and ^{125}I-labeled abnormal HF by trypsin and generation of the prekallikrein-activating activity was studied by simultaneously following the kinetics of cleavage of HF by trypsin and the rate of

generation of prekallikrein-activating activity from HF. A mixture of 1 μl ^{131}I-normal HF and 60 μl normal HF (2 μg) was activated with 8 μl trypsin (80 ng, twice crystallized, salt-free, Worthington Biochemical Corp., Freehold, N. J.) at 37°C in a 10 × 75-mm polystyrene tube. At intervals, the action of trypsin was blocked by 16 μl lima bean trypsin inhibitor (LBTI, 160 ng, Worthington Biochemicals); the action of activated HF was not inhibited by this concentration of LBTI (14). A 15-μl aliquot was then tested for prekallikrein-activating activity by incubating at 0°C for 5 min with 300 μl of 1:5 diluted HF-deficient plasma. A 50-μl portion of this mixture was tested for plasma kallikrein activity by incubating with 2 ml 0.2 mM H-D-proline phenylalanine-arginine-PNA (S-2302, Kabi Diagnostics, Stockholm, Sweden) at 37°C for 10 min. The reaction was terminated by addition of 0.2 ml glacial acetic acid and absorbance at 405 nm was read. Exactly the same experiment was performed using a mixture of 1 μl ^{125}I-abnormal HF and 60 μl abnormal HF (2 μg). To examine the cleavage of HF by trypsin, 27-μl aliquots of the trypsin digests of both normal and abnormal HF were combined at the indicated time and subjected to SDS-PAGE as described above.

[^{3}H]Diisopropyl phosphorofluoridate (DFP) incorporation into trypsin-cleaved normal and abnormal HF was tested by measuring the uptake of [^{3}H]DFP by the normal and abnormal HF after the treatment with trypsin. The conditions for the cleavage of HF by trypsin were identical to those described in the preceding section. 4 μg of normal or abnormal HF was cleaved with 160 ng trypsin in a total volume of 129 μl at pH 7.5 at 37°C for 10 min. After the action of trypsin had been blocked by addition of 32 μl LBTI (320 ng), the mixture was incubated at 37°C for 2 h with 1 mM DFP containing 100 μCi of [^{3}H]DFP (6.5 Ci/mmol, Amersham, Arlington Heights, Ill.) in a total volume of 183 μl. The samples were then extensively dialyzed and subjected to SDS-PAGE after reduction with 2-mercaptoethanol. Control experiments were performed in which HF was first mixed with LBTI and then with trypsin, or HF was replaced by BS. The gels were cut into 2-mm segments and dissolved in 1% periodic acid (15); after the addition of Aquasol scintillator (New England Nuclear, Boston, Mass.), ^{3}H was measured in a liquid scintillation spectrometer (Nuclear Chicago).

RESULTS

Isolation of HF from a CRM$^+$ Hageman trait plasma. Abnormal HF was isolated from ~300 ml citrated plasma of an individual with CRM$^+$ Hageman trait by sequential column chromatography on DEAE-Sephadex A-50, lysine-Sepharose, DEAE-Sephadex A-50, SP-Sephadex C-50, and Sephadex G-150. The last Sephadex G-150 column was used to remove trace amounts of contaminating high molecular-weight proteins from HF. During isolation, localization of immunoreactive HF (HF antigen) was identified by a solid-phase RIA. Approximately 400 μg of purified abnormal HF was obtained (10% yield); its specific activity was 23 U immunoreactive HF per milligram protein by RIA and 0 U/mg protein in a clot-promoting assay. Normal HF was purified by the same procedure from citrated plasma of a single donor; the purified protein had the specific activity of 22 U immunoreactive HF per milligram protein and 73 U/mg protein in the clot-promoting assay. The discrepancy in the specific activities of normal HF between the immunoassay and the clotting assay has been previously noted and is probably due to overestimation of the HF activity of the purified preparation compared to that in whole plasma (3). The elution patterns of abnormal HF from the ion-exchange and gel filtration columns were very similar to those of normal HF. Two separate lots of abnormal HF, isolated on different occasions, were used in the following experiments and gave essentially identical results.

Properties of abnormal HF. A single sharp protein band was observed by SDS-PAGE of both normal and abnormal HF before and after reduction with 2-mercaptoethanol (Fig. 1). These bands were also positively stained with periodic acid-Schiff. The apparent molecular weight estimated by this technique was 80,000 for both normal and abnormal HF. These data indicate that abnormal HF is a glycoprotein consisting of a single polypeptide chain with the same molecular weight as normal HF.

To show that the band on SDS gels were indeed HF, purified normal and abnormal HF were electrophoresed on nondenatured polyacrylamide gels. The protein band of normal HF appeared at the same place as the HF clot-promoting activity and HF antigen (Fig. 2 A). Similarly, the protein band of abnormal HF was detected at the same place as HF antigen (Fig. 2 B).

Upon immunodiffusion, purified abnormal HF showed a line of complete identity with purified normal HF against anti-HF serum, and upon immunoelectrophoresis, abnormal HF had the same electrophoretic mobility as its normal counterpart.

The amino acid compositions of abnormal HF was similar to that of normal HF (Table I). Isoelectric focusing of both normal and abnormal HF demonstrated three distinct protein bands, with pI 6.5 ~ 7.1 (Fig. 3). Each protein band appeared to be associated with HF antigen. These amino acid analysis and pI data are essentially similar to those in previous reports (16–18).

Although all these data suggest that abnormal HF is indistinguishable from normal HF by physicochemical and immunological means, abnormal HF had essentially no clot-promoting function. 12.5 ng of purified normal HF clotted HF-deficient plasma in 76.4 s, and 4,200 ng (336-fold more) abnormal HF, in 250 s (Table II). The addition of 210 ng abnormal HF to 12.5 ng normal HF did not inhibit the clot-promoting activity of normal HF, which suggests that there was no inhibitor present in abnormal HF.

Cleavage of normal and abnormal HF in the contact activation of plasma. It has been shown that normal human HF, a single chain polypeptide of 80,000 mol wt, undergoes proteolytic cleavage to form 52,000- and 28,000-mol wt fragments upon contact activation in plasma (13). Plasma kallikrein has been assumed

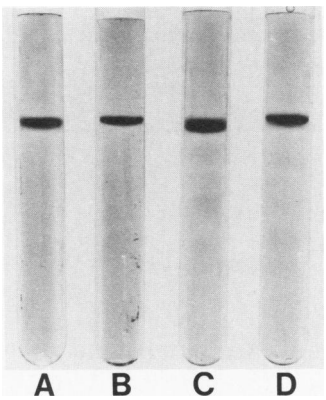

FIGURE 1 SDS-PAGE of purified normal (A and B) and abnormal (C and D) HF. Gels A and B are 5 μg of normal HF before and after reduction; gels C and D are 5 μg of abnormal HF before and after reduction, respectively. The anode was at the bottom of the gel.

TABLE I
Amino Acid Composition

	Normal HF	Abnormal HF
	%	%
Lysine	3.7	3.7
Histidine	4.7	4.8
Arginine	6.8	6.3
Aspartic acid	6.5	6.7
Threonine	4.9	4.5
Serine	5.9	5.3
Glutamic acid	11.3	11.6
Proline	9.8	9.8
Glycine	8.6	8.9
Alanine	9.1	9.5
Half-cystine	7.4	7.1
Valine	5.3	5.0
Methionine	0.4	0.7
Isoleucine	1.8	2.0
Leucine	8.4	8.8
Tyrosine	2.8	2.6
Phenylalanine	2.6	2.6

These values are the average of duplicate determinations.

to be primarily responsible for cleavage of HF (13). We have examined whether abnormal HF is fragmented in the same way as normal HF. The cleavage of both normal and abnormal HF has been studied in normal plasma and in a CRM$^+$ Hageman trait plasma shaken in glass.

Normal plasma containing both ^{131}I-labeled normal HF and ^{125}I-labeled abnormal HF was exposed to glass for varying periods of time and was then subjected to SDS-PAGE before and after reduction with 2-mercaptoethanol. The patterns of radioactivity of ^{131}I and ^{125}I on gels were then examined (Fig. 4). At zero time, a single large peak representing a native molecule of 80,000 mol wt was seen in both normal and abnormal HF. After 5 min, the initial peak of both normal and abnormal HF decreased and new peaks appear at 52,000 and 30,000 mol wt in both reduced and unreduced samples, although more cleavage was found in the presence of the reducing agent (Fig. 4C vs. 4D). These data suggest some scission occurred within a disulfide loop yielding fragments connected by disulfide linkages. Under these conditions, the cleavage profile of abnormal HF was identical to that of normal HF and is in good agreement with those previously reported for normal HF (13).

When a CRM$^+$ Hageman trait plasma containing ^{125}I-abnormal HF was exposed to glass, there was no cleavage of abnormal HF detected. However, normal fragmentation of abnormal HF was found when a mixture of ^{125}I-abnormal HF and nonradioactive normal HF in a CRM$^+$ plasma was added to glass. Similarly, normal scission was observed when ^{125}I-normal HF in a CRM$^+$ plasma was exposed to glass.

Cleavage of normal and abnormal HF by trypsin and generation of prekallikrein activator activity.

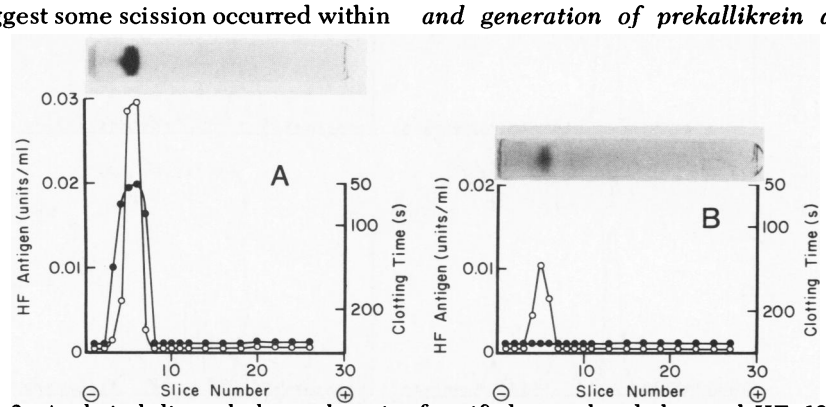

FIGURE 2 Analytical disc gel electrophoresis of purified normal and abnormal HF. 12 μg of purified normal HF (A) or 4 μg of abnormal HF (B) was electrophoresed in 6% polyacrylamide as shown on the top panel. Protein was identified by staining with Coomassie Blue. A duplicate gel was sliced into 2-mm segments and assayed for HF clot-promoting activity and antigen as shown in the lower panel. ○ —— ○ HF antigen; ● —— ● HF clotting activity.

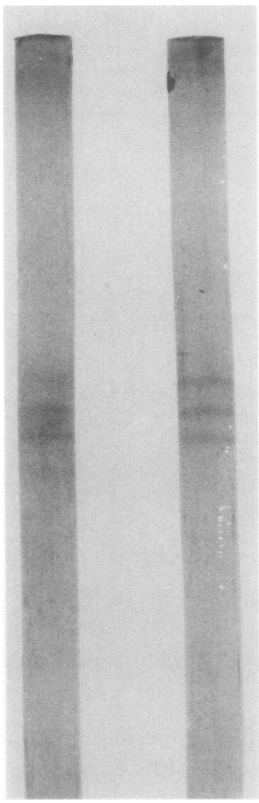

FIGURE 3 Isoelectric focusing of 5 μg each of purified normal (right) and abnormal (left) HF. The anode was at the bottom of the gel.

TABLE II
Clot-promoting Activity of Purified Normal and Abnormal HF

Test sample	Clotting time
ng	*s*
Normal HF, 50	55.9
Normal HF, 25	65.9
Normal HF, 12.5	76.4
Abnormal HF, 4,200	250.0
Abnormal HF, 420	306.2
Abnormal HF, 42	>350.0
Normal HF, 12.5 + Abnormal HF, 210	75.0
Buffer	>350.0

0.1 ml of various concentrations of purified normal or abnormal HF was assayed for HF clot-promoting activity as described in Methods.

Trypsin is known to cleave normal HF into 52,000-, 40,000-, and 28,000-mol wt fragments and to generate prekallikrein-activating properties (17). The 28,000-mol wt fragment is a serine protease and carries prekallikrein-activator activity (19–21). When ^{131}I-labeled normal HF and ^{125}I-labeled abnormal HF were digested with trypsin, both underwent an identical pattern of scission into 52,000-, 40,000-, and 29,000-mol wt fragments, as determined by SDS-PAGE (Fig. 5). Potent prekallikein-activator properties were generated when normal HF was digested by trypsin, but were not observed when the abnormal HF was similarly treated; in these experiments, the amidolytic properties of trypsin were blocked by the addition of LBTI before assay. Thus, the failure to generate the enzymatic activity is not due to the lack of the scission.

Incorporation of [^{3}H]DFP into trypsin-cleaved normal and abnormal HF. When normal HF was cleaved

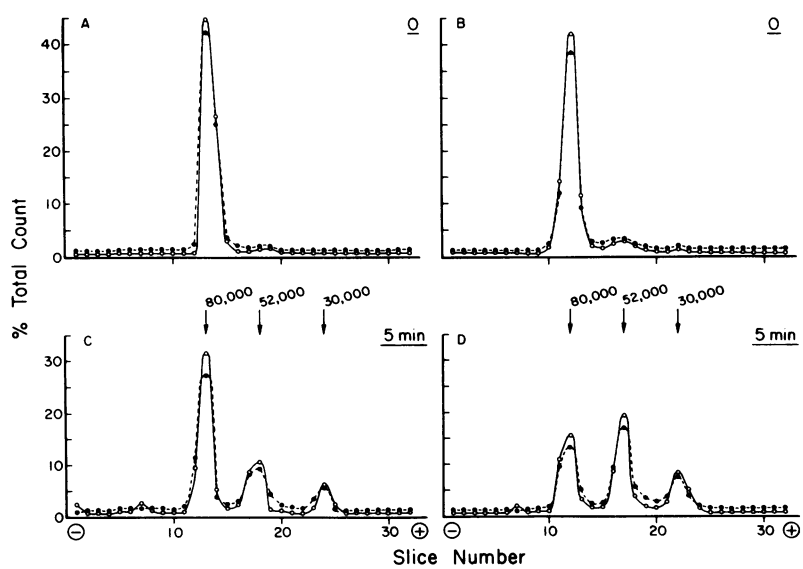

FIGURE 4 SDS-PAGE of ^{131}I-normal HF and ^{125}I-abnormal HF in normal plasma after exposure to glass. (A) 0 time, nonreduced; (B) 0 time, reduced; (C) 5 min, nonreduced; (D) 5 min, reduced. ○ —— ○ ^{131}I; ● - - - ● ^{125}I.

and activated by trypsin, a 29,000-mol wt fragment incorporated [^{3}H]DFP (Fig. 6). In contrast, a 29,000-mol wt piece from the abnormal HF failed to take up [^{3}H]-DFP under the same conditions, although trypsin had cleaved the abnormal HF into 52,000- and 29,000-mol wt fragments, as shown in the preceeding section. In that the 29,000-mol wt part of the molecule has been shown to contain an active site serine in both human and bovine HF (22–24), these data indicate that the molecular defect of the abnormal HF is localized around an active center serine residue in the 29,000-mol wt light chain.

DISCUSSION

In the present study, an abnormal HF was isolated from plasma of a CRM$^+$ Hageman trait subject. The product was homogeneous by several criteria, including SDS-PAGE, alkaline disc gel electrophoresis, and immunoelectrophoresis. The abnormal HF showed complete identity with normal HF upon immunodiffusion against anti-HF serum and had the same specific antigenicity in RIA as normal HF. Thus, the normal and abnormal HF were immunologically indistinguishable. Nonetheless, the abnormal HF was essentially devoid of the clot-promoting activity. We sought to investigate the nature of the defect in this abnormal HF.

Normal HF is a single chain polypeptide of 80,000 mol wt with at least one internal disulfide bridge (17),

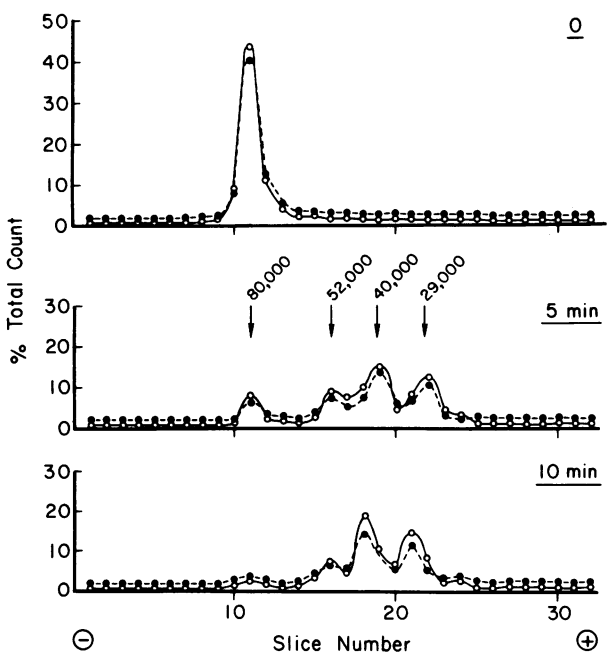

FIGURE 5 SDS-PAGE of ^{131}I-normal HF and ^{125}I-abnormal HF treated with trypsin for varying periods of time. ○ —— ○ ^{131}I; ● - - - ● ^{125}I.

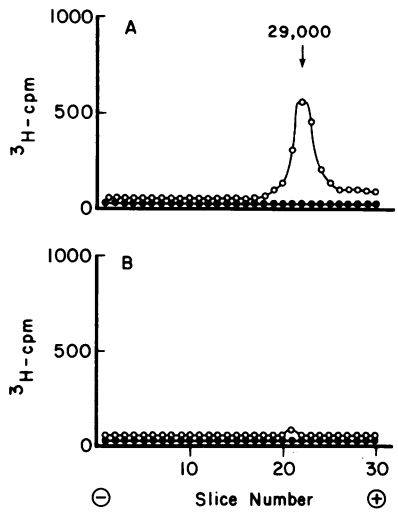

FIGURE 6 Incorporation of [^{3}H]DFP into trypsin-cleaved normal and abnormal HF. 4 μg of normal (A) or abnormal (B) HF was cleaved by 160 ng trypsin and, after trypsin was inhibited by 320 ng LBTI, the mixture was incubated at 37°C for 2 h with 1 m M DFP containing 100 μCi of [^{3}H]DFP. After dialysis, the samples were analyzed on SDS-PAGE in the presence of 2-mercaptoethanol. ○ —— ○ HF + trypsin → LBTI; ● —— ● HF + LBTI → trypsin.

and is a zymogen of a serine protease (23–25). When blood comes into contact with such negatively charged surfaces as glass and kaolin, HF, prekallikrein, plasma thromboplastin antecedent (Factor XI), and high molecular-weight kininogen are all adsorbed to the surface and HF becomes enzymatically active. The initial event that triggers the activation of HF is not yet clear, but it has been postulated that a conformational change in HF molecule upon binding (26), a low degree of inherent enzymatic activity in the HF zymogen (23), or the interaction of HF with prekallikrein on a surface (a substrate-induced catalysis) (27) may activate a few molecules of HF. Autoactivation of HF has also been suggested as a trigger (28, 29). In any event, once HF is activated, it in turn activates prekallikrein to kallikrein that further activates HF (reciprocal activation) (30). During these reactions, HF undergoes specific limited proteolytic cleavage, yielding 52,000 mol wt (heavy chain) and 28,000-mol wt fragments (light chain) (13). Cleavage seems to occur at two specific sites in the molecule that are inside and outside of a disulfide bridge. The 28,000-mol wt part of the molecule appears to contain an active serine site, while the 52,000-mol wt part is related to the binding to surfaces (13). Therefore, at least three possibilities may be considered as responsible for the defect in the abnormal nonfunctional HF: (*a*) an inability to bind to a surface, (*b*) an inability to be cleaved at specific sites, or (*c*) a defective active center in the cleaved light chain.

TABLE III
Summary of Properties of an Abnormal HF

	Normal HF	Abnormal HF
Molecular weight	80,000	80,000
Periodic acid-Schiff staining	+	+
Amino acid composition	Similar	
pI	6.5 ~ 7.1	6.5 ~ 7.1
Immunodiffusion	Complete identity	
Binding to surfaces	+	+
Limited proteolysis in the contact activation of plasma or by trypsin	+	+
[^{3}H]DFP incorporation	+	–
Enzymatic activities*	+	–

* Clot-promoting and prekallikrein-activating activities.

Table III summarizes some characteristics of this abnormal HF. Both normal and abnormal HF were glycoproteins consisting of a single polypeptide chain with an apparent molecular weight of 80,000. The amino acid composition and pI of abnormal HF were similar to those of normal HF and are in agreement with those reported previously for the normal molecule (16–18). It should be noted, however, that an amino acid analysis of protein cannot detect a single amino acid substitution. The abnormal HF was as readily adsorbed to negatively charged surfaces as normal HF (2), and underwent an identical pattern of scission after contact activation in normal plasma or tryptic digestion. Therefore, the functional defect in this abnormal HF did not appear to be due to the failure to adsorb to surfaces or to the absence of the specific cleavages. Very small difference in the size of fragments, however, might not have been detected by the technique used. Furthermore, it should be recognized that the abnormal HF showed no cleavage when exposed to glass in the CRM$^+$ Hageman trait plasma from which it had been isolated. This was probably related to the defective reciprocal activation of HF and prekallikrein (30) in the CRM$^+$ Hageman trait plasma in which kallikrein activation was absent. This observation appears to support the hypothesis that HF is the first molecule to be activated in the interaction of HF, prekallikrein, and surfaces (1, 28, 29).

We investigated the possibility that the defect in the abnormal HF was at the active site in the light chain by using radioactive DFP, which specifically reacts with an active site serine residue. Upon the scission by trypsin, the abnormal HF did not incorporate [^{3}H]-DFP into its 29,000-mol wt fragment, whereas normal HF did. These data indicate that the defect in this abnormal HF resided at or near an active site serine residue in the light chain of the molecule.

The importance of a histidine as well as a serine residue in the catalytic function has been recognized in some serine proteases such as trypsin and plasmin (31, 32). Both trypsin and plasmin contain a single DFP-sensitive serine and a single L-1-chloro-3-tosylamido-7-amino-2-heptanone (TLCK)-sensitive histidine at their active center. The modification of a histidine residue in the active center of trypsin abolished the incorporation of DFP into an active site serine (31). Thus, the failure of abnormal HF to take up [^{3}H]-DFP may have been due to a substitution at either a serine or histidine residue assuming that activated HF is a trypsinlike enzyme. Although it would be interesting to study the incorporation of radioactive TLCK into normal and abnormal HF, radioactive TLCK is not available at the present time.

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