Functional Characterization of an Abnormal Factor XII Molecule (F XII Bern)

By W.A. Wuillemin, I. Huber, M. Furlan, and B. Lämmlle

An 18-year-old healthy woman was found to have cross-reacting material (CRM)-positive factor XII (F XII) deficiency. F XII clotting activity was less than 0.01 U/mL, whereas F XII antigen was 0.11 U/mL. An F XII inhibitor was excluded. To partially characterize the molecular defect of the abnormal F XII, immunologic and functional studies were performed on the proposita's plasma. The abnormal F XII was a single chain molecule with the same molecular weight (80 Kd) and the same isoelectric points (pL, 5.9 to 6.8) as normal F XII. Dextran sulfate activation of the proposita's plasma showed no proteolytic cleavage of F XII even after 120 minutes, whereas F XII in pooled normal plasma, diluted 1:10 with CRM-negative F XII-deficient plasma, was completely cleaved after 40 minutes. Adsorption to kaolin was identical for both abnormal and normal F XII. In the presence of dextran sulfate and exogenous plasma kallikrein, the abnormal F XII was cleaved with the same rate as normal F XII. However, kallikrein-cleaved abnormal F XII was not able to cleave factor XI and plasma prekallikrein, in contrast to activated normal F XII. Thus, these studies show that the functional defect of this abnormal F XII, denoted as F XII Bern, is due to the lack of protease activity of the kallikrein-cleaved molecule. Therefore, the structural defect is likely to be located in the light chain region of F XII, containing the enzymatic active site.

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HUMAN BLOOD coagulation factor XII (F XII), also called Hageman factor, is a plasma glycoprotein that is functionally deficient in individuals with Hageman trait. F XII is a zymogen of a serine protease with an approximate molecular weight (mol wt) of 80 Kd and is present in human plasma at a concentration of 15 to 47 µg/mL. F XII appears to play an important role in blood coagulation, fibrinolysis, and kinin generation. When plasma comes into contact with negatively charged surfaces such as glass, kaolin, or dextran sulfate, F XII is bound to the surface and undergoes limited proteolysis by plasma kallikrein, resulting in two active enzyme forms, α-F XIIa and β-F XIIa. α-F XIIa is composed of two disulfide-linked polypeptide chains, a heavy chain with a mol wt of 52 Kd and a light chain with a mol wt of 28 Kd. The heavy chain contains the surface binding site and is composed of 353 amino acid residues, while the light chain, made up of 243 amino acid residues, contains the active site triad of serine proteases. β-F XIIa consists of two disulfide-linked chains, a light chain with a mol wt of 2 Kd and a heavy chain with a mol wt of 28 Kd, containing the catalytic domain.

The majority of individuals with congenital F XII deficiency lack immunologically identifiable F XII. Very rare subjects have been reported whose plasma contained detectable amounts of nonfunctional but immunologically identifiable F XII. Because the plasmas of these individuals contain cross-reacting material (CRM), they have been called CRM-positive variants of F XII deficiency. The study of these dysfunctional F XII molecules provides a unique opportunity to examine the structure-function relationship of this protein.

The present study was undertaken to characterize the functional defect of a F XII variant, denoted as F XII Bern, which had been recently discovered in a study on 74 subjects from 14 Swiss families with congenital F XII deficiency. The results indicate that the functional abnormality is due to the lack of protease activity. Thus, the structural defect is probably located on the light chain of F XII, affecting the enzymatic active site.

MATERIALS AND METHODS

Plasma samples. Informed consent for venipuncture was obtained from the proposita and her family. Blood samples (9 mL) were obtained by clean venipuncture with plastic syringes (Monovette, Sarstedt, Nümbrecht, Germany) containing 1 mL 0.106 mol/L trisodium citrate. Plasma samples obtained by twice centrifuging at 1,500g for 10 minutes at room temperature were handled using plastic ware, and were stored in polypropylene tubes at -70°C until use. A normal human plasma pool (NHP) from 35 healthy male volunteers was stored in small aliquots in liquid nitrogen. NHP was used as standard for measurement of the clotting activity of factor XI (F XI:C), high molecular weight kininogen, prekallikrein, and F XII (F XII:C) as well as for measurement of F XII antigen (F XII:Ag). NHP was defined to contain 1 U/mL of clotting factor activity and of antigen concentration, respectively. Pooled F XII-deficient plasma was prepared by mixing equal volumes of plasma obtained from 17 individuals congenitally deficient in F XII. All 17 subjects had F XII:C less than 0.01 U/mL and no detectable F XII:Ag in immunoblot assays (<0.01 U/mL). Plasmas congenitally deficient in F XI, high molecular weight kininogen, or prekallikrein were purchased from George King Biomedical (Overland Park, KS). One volume of NHP was mixed with 9 volumes of pooled F XII-deficient plasma to obtain a plasma mixture containing 0.1 U/mL of normal F XII. This plasma mixture was used in all experiments for comparison with the proposita’s plasma.

Reagents. Nitrocellulose membrane filters ( pore size, 0.2 µm) were from Schleicher & Schuell (Keene, NH). Dextran sulfate (mol wt 50 Kd, sulfur content 17%), CNBr-activated Sepharose 4B and 2-D Pharmalyte 3-10 were from Pharmacia (Uppsala, Sweden). Microniced kaolin was from British Drug House (Poole, UK). The chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S-2302) was from KabiVitrum (Stockholm, Sweden). Soybean trypsin inhibitor (SBTI) was from Merck (Darmstadt, Germany). The anti-F XII antiserum used was a polyclonal goat antihuman F XII antigen (F XII:Ag). NHP was defined to contain 1 U/mL of F XII:C as well as for measurement of F XII antigen (F XII:Ag).

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Submitted September 25, 1990; accepted April 8, 1991.

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tor and human α2-antiplasmin were from Behring (Marburg, Germany).

**Purified proteins.** F XII was purified from NHP according to Griffin and Cochrane to a specific clotting activity of 61 U/mg. Prekallikrein was purified as described by Van der Graaf et al. with a minor modification, and had a specific clotting activity of 212 U/mg. Plasma kallikrein was prepared by incubating prekallikrein with B-F XIIa and was stored at -70°C in TBS (10 mmol/L Tris, 140 mmol/L NaCl, pH 7.4). F XII was isolated as described by Bouma et al. and had a specific clotting activity of 212 U/mg. Purified F XII, prekallikrein, and F XI were stored at -70°C in storage buffer (4 mmol/L acetate, 2 mmol/L acetic acid, 150 mmol/L NaCl, pH 5.3) and were radiolabeled with 125I by a chloramine T method. The radioiodinated proteins had a specific radioactivity of about 40 μCi/μg protein (125I-F XII and 125I-prekallikrein) and about 20 μCi/μg protein (125I-F XI), respectively. They were stored in a buffer containing 50 mmol/L acetate, 1 mmol/L barbital, 1 mg/mL BSA, pH 7.35. Twenty microliters of the diluted test sample and 20 μL of Neothromtin (consisting of a vegetable phospholipid and ellagic acid; Behring) for 4 minutes with a buffer containing 47.4 mmol/L acetate, 28.55 mmol/L barbital, 1 mg/mL BSA, pH 7.35. Twenty microliters of the respective congenitally deficient plasma were incubated with 20 μL of the diluted test sample and 20 μL of Neothromtin (consisting of a vegetable phospholipid and ellagic acid; Behring) for 4 minutes. The kaolin reaction mixtures containing 125I-F XII or 125I-prekallikrein were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by electrotransfer onto nitrocellulose membranes and autoradiography.

**Immunoblotting of F XII.** Immunoblotting of F XII and its proteolytic fragments was performed as previously reported. For qualitative and quantitative immunoblotting as well as for dot immunobinding assays, a recently described immunodetection system was used. In short, F XII immobilized on nitrocellulose filters was detected by polyclonal goat anti-F XII antibodies (the presence or absence of exogenous plasma kallikrein). Sixty-seven microliters of proposita’s plasma or (as a control) pooled F XII-deficient plasma containing 10% (vol/vol) NHP, respectively, were mixed with 60 μL TBS, containing 1 mg/mL BSA, and incubated at 37°C with 20 μL kaolin suspension in TBS (giving a final kaolin concentration of 0.1 to 5 mg/mL), or with 20 μL TBS as a control. Incubation was stopped after various time intervals (0.5 to 5 minutes) by centrifuging reaction mixtures for 30 seconds. Twenty microliters of the supernatant were removed for measuring unbound F XII:Ag by dot immunobinding. Cleavage of F XI and prekallikrein in kaolin-activated plasma (in the presence or absence of exogenous plasma kallikrein). The mother of the proposita showed a slightly prolonged activated partial thromboplastin time of 12 seconds.
FACTOR XII BERN

Table 1. Coagulation Screening Tests, Clotting Activity, and Antigen Concentration of Coagulation Factors in the Plasma of the Proposita and Her Family Members.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proposita</th>
<th>Mother</th>
<th>Brother</th>
<th>“Father”</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated partial thromboplastin time (sec)§</td>
<td>375</td>
<td>72</td>
<td>55</td>
<td>55</td>
<td>40-60</td>
</tr>
<tr>
<td>Prothrombin time (Quick %)</td>
<td>78</td>
<td>83</td>
<td>75</td>
<td>100</td>
<td>70-130</td>
</tr>
<tr>
<td>F XII:C (U/mL)</td>
<td>&lt;0.01</td>
<td>0.17</td>
<td>0.69</td>
<td>0.97</td>
<td>0.61-1.45</td>
</tr>
<tr>
<td>F XII:Ag (U/mL)†</td>
<td>0.09</td>
<td>0.20</td>
<td>0.77</td>
<td>0.83</td>
<td>0.71-1.44</td>
</tr>
<tr>
<td>F XII:Ag (U/mL)§</td>
<td>0.11</td>
<td>0.21</td>
<td>0.67</td>
<td>0.73</td>
<td>—</td>
</tr>
<tr>
<td>F XI:C (U/mL)</td>
<td>0.83</td>
<td>0.73</td>
<td>0.72</td>
<td>0.90</td>
<td>0.65-1.40</td>
</tr>
<tr>
<td>High mol wt kininogen clotting activity (U/mL)</td>
<td>0.92</td>
<td>0.95</td>
<td>1.05</td>
<td>0.81</td>
<td>0.65-1.20</td>
</tr>
<tr>
<td>F VIII:C (U/mL)</td>
<td>0.63</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.50-1.50</td>
</tr>
<tr>
<td>F IX:C (U/mL)</td>
<td>0.78</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.70-1.30</td>
</tr>
</tbody>
</table>

*Dot immunobinding assay.
†Radial immunodiffusion.
‡Quantitative immunoblotting.
§Kaolin-inosithin reagent.

Table 1. Coagulation Screening Tests, Clotting Activity, and Antigen Concentration of Coagulation Factors in the Plasma of the Proposita and Her Family Members.

The presumed father and the brother of the proposita had normal levels of the measured clotting factors. Neither the proposita nor her family members had a history of excessive bleeding or thromboembolic events.

Effect of proposita’s plasma on NHP. No inhibitory activity of the proposita’s plasma on normal plasma activated partial thromboplastin time or normal plasma F XII:C level was found when plasma mixtures (50% NHP and 50% proposita’s plasma, vol/vol) were assayed either before or 1 hour after incubation of the plasma mixtures at 37°C. Thus, the proposita’s plasma represents a CRM-positive F XII deficiency. The abnormal F XII molecule was tentatively called F XII Bern after the city where this plasma was studied. Purification of the abnormal F XII was not possible due to the limited amount of plasma available.

Isoelectric focusing of F XII in proposita’s plasma and in NHP. Proposita’s plasma and pooled CRM-negative F XII deficient plasma containing 10% (vol/vol) of NHP were focused on polyacrylamide gels (pH range, 3 to 10). F XII was detected by autoradiography after electrotransfer of the proteins from the gels onto nitrocellulose membranes and reaction with anti-F XII antiserum followed by 125I-F XII overlay. The isoelectric focusing pattern of F XII Bern and normal F XII were identical (Fig 1), showing isoelectric points between 5.9 and 6.8.

Dextran sulfate activation of plasma. Dextran sulfate activation of the proposita’s plasma for up to 2 hours was not accompanied by proteolytic F XII cleavage (Fig 2A, left panel), whereas normal F XII was cleaved upon dextran sulfate activation of F XII-deficient plasma containing 10% (vol/vol) of NHP (Fig 2A, right panel). Under reducing conditions, single-chain normal plasma F XII (mol wt 80 Kd) disappeared and concomitantly α-F XIIa heavy chain (mol wt 52 Kd) became visible (Fig 2A, right panel). The F XII antisera did not detect the light chain of α-F XIIa (mol wt 28 Kd). The appearance of a F XII fragment at about 50 Kd mol wt under non-reducing conditions demonstrated formation of β-F XIIa. The high mol wt bands at about 195 and 145 Kd appearing during contact activation of NHP were apparently due to complexes of α-F XIIa with protease inhibitors. To investigate which protease inhibitors were involved in α-F XIIa binding, dextran sulfate-activated NHP was incubated with antisera to protease inhibitors. The immunoprécipitates and supernatants were subjected to immunoblotting of F XII (Fig 3). Anti-C1 inhibitor antiserum and anti-α,-antiplasmin antiserum removed the bands at 195 and 145 Kd, respectively, demonstrating that these complexes were composed of α-F XIIa-C1 inhibitor (195 Kd) and α-F XIIa-α,-antiplasmin (145 Kd).

Dextran sulfate activation of the mother’s plasma (F XII:C = 0.17 U/mL, and F XII:Ag = 0.20 U/mL) resulted in normal cleavage of F XII when compared with CRM-negative F XII-deficient plasma containing 20% (vol/vol) of NHP (data not shown), indicating the presence of a normal F XII molecule at reduced concentration in the mother’s plasma.

There was no generation of plasma kallikrein during dextran sulfate activation of proposita’s plasma as assessed by measuring the amidolytic activity against S-2302. However, dextran sulfate activation of F XII-deficient plasma containing 10% (vol/vol) NHP resulted in progressive generation of amidolytic activity against S-2302 (Fig 2B).
the reaction mixtures were removed and either subjected to immuno-
negative F XII-deficient plasma containing 10% (vol/vol) NHP. Equal
volumes of dextran sulfate-activated proposita’s plasma were
incubated on ice. After various time intervals, aliquots of
dextran sulfate-activated proposita’s plasma
Generation of amidolytic activity against
XI1 immunoblotting analysis of unreduced and reduced samples of
“0
sulfate activation of proposita’s plasma
Mol wts \( \times 10^3 \) (Kd) of prestained protein standards are indicated.

tation mixtures, supematants and immunoprecipitates were subjected
to immunoblotting of F XII. Samples contained 1 \( \mu \)L of dextran
sulfate-activated NHP (lane 1); 1 \( \mu \)L of supernatant of dextran
sulfate-activated NHP incubated with anti-C1-inhibitor antiserum
(lane 2); immuno-precipitate of 2 \( \mu \)L of dextran sulfate-activated NHP
incubated with anti-C1-inhibitor antiserum (lane 3); 1 \( \mu \)L of superna-
tant of dextran sulfate-activated NHP incubated with anti-\( \alpha \)-antiplasmin antiserum
(lane 4); immuno-precipitate of 5 \( \mu \)L of dextran
sulfate-activated NHP incubated with anti-\( \alpha \)-antiplasmin antiserum
(lane 5). Mol wts \( \times 10^3 \) (Kd) are indicated.

There are at least three possibilities that may account for
the lack of F XII activation during dextran sulfate exposure
of the proposita’s plasma: (1) inability of the abnormal F
XII to bind to a negatively charged surface; (2) impaired
proteolytic cleavage by plasma kallikrein; and (3) defective
proteolytic activity against its substrates, prekallikrein and/or
XI. These three possibilities were tested separately.

Kaolin binding of F XII. Proposita’s plasma and F
XII-deficient plasma containing 10% (vol/vol) NHP were
incubated with various amounts of kaolin for different time
intervals. The kinetics of F XII binding to kaolin was similar
for F XII Bern and normal F XII (Fig 4).

Cleavage of F XII by exogenous plasma kallikrein. Propos-
ita’s plasma or F XII-deficient plasma containing 10% (vol/vol) NHP was exposed to dextran sulfate and plasma
kallikrein (final concentration, 12 \( \mu \)g/mL) at 4°C. As shown
in Fig 5, the time course of F XII cleavage was similar for
both F XII Bern and normal F XII. However, the complex-
es of \( \alpha \)-F XIIa with C1 inhibitor and \( \alpha \)-antiplasmin were not observed in the proposita’s plasma, despite
normal levels of these two serpins in the proposita’s plasma; the concentrations of \( \alpha \)-antiplasmin (102%) and C1 inhibi-
tor (132%), and also of the plasma kallikrein inhibitor,
\( \alpha \)-macroglobulin (2.9 mg/mL), in the proposita’s plasma
were similar to the respective values in the F XII-deficient
plasma containing 10% NHP (101%, 97%, and 2.4 mg/mL,
respectively).

Cleavage of F XI and prekallikrein by activated F XII in
plasma. Proposita’s plasma or F XII-deficient plasma containing 10% (vol/vol) NHP was incubated with kaolin for 2 hours at 37°C. Under these conditions, normal F XII was completely cleaved in contrast to F XII Bern (Fig 6A). When exogenous plasma kallikrein (final concentration, 100 \( \mu \)g/mL) had been added to the kaolin-plasma mixtures, both normal and abnormal F XII were almost completely

A

B

Fig 2. Dextran sulfate activation of proposita’s plasma and CRM-
negative F XII-deficient plasma containing 10% (vol/vol) NHP. Equal
volumes of dextran sulfate (25 \( \mu \)g/mL in water) and of the respective
plasma were incubated on ice. After various time intervals, aliquots of
the reaction mixtures were removed and either subjected to immuno-
blotting of F XII or tested for amidolytic activity against
S-2302. (A) F
XII immunoblotting analysis of unreduced and reduced samples of
dextran sulfate-activated proposita’s plasma (F XII Bern) and dextran
sulfate-activated F XII-deficient plasma containing 10% NHP (NHP).
Mol wts \( \times 10^3 \) (Kd) of prestained protein standards are indicated. (B) Generation of amidolytic activity against S-2302 during dextran
sulfate activation of proposita’s plasma (●●●) and of F XII-deficient
plasma containing 10% NHP (△△△).
DISCUSSION

Hereditary deficiencies of coagulation factors are caused either by the absence of a clotting factor or by the synthesis of a defective molecule. F XII Bern is one of four dysfunctional F XII variants so far described. Abnormal F XII molecules provide an opportunity to examine the structure-function relationship of this plasma protein.

The proposita described here has been recently detected to have CRM-positive F XII deficiency. The presence of an abnormal F XII molecule was now confirmed using three different methods for F XII:Ag measurement, all showing a F XII:Ag value of about 10% of that in NHP, whereas no F XII clotting activity was detectable (Table 1). Coagulation assays on mixtures of proposita’s plasma and NHP ruled out an acquired or inherited F XII inhibitor. As most subjects with F XII:C deficiency, the proposita appeared to be clinically asymptomatic. Studies of the family members indicated that the mother had about 0.2 U/mL of both F XII:C and F XII:Ag, whereas a brother showed normal F XII:C and F XII:Ag values (Table 1). This finding suggests that the mother possesses one normal gene for F XII and one gene that is not expressed. The proposita appears to have two abnormal F XII genes, one not expressing any gene product and one with impaired production of a dysfunctional F XII molecule. However, the mode of inheritance of the variant F XII molecule could not be established by family analysis, because the putative father of the proposita had normal F XII:C and F XII:Ag values.

Purification of this abnormal protein (plasma concentration about 2.5 μg/mL) was not possible due to the limited amount of plasma available. Functional characterization of this abnormal F XII was performed therefore with whole plasma and by comparing proposita’s plasma with pooled CRM-negative F XII-deficient plasma containing 10% of NHP.

An identical apparent mol wt (about 80 Kd) of F XII Bern and normal F XII was observed before and after reduction in immunoblots (Fig 2A). The isoelectric focusing pattern of normal and dysfunctional F XII was identical, showing several distinct isoelectric forms with pIs between 5.9 and 6.8 (Fig 1), in agreement with other reports on purified normal F XII. These data indicate that F XII Bern is a single-chain molecule with similar mol wt and PI as normal F XII.

Dextran sulfate activation of the proposita’s plasma did not result in F XII cleavage, and no plasma kallikrein activity was generated, in contrast to dextran sulfate activation of NHP diluted in CRM-negative F XII-deficient plasma (Fig 2A and B).

When plasma comes into contact with a negatively charged surface, F XII, kallikrein, F XI, and high...
molecular weight kininogen are adsorbed to the surface and F XII becomes highly susceptible to proteolytic cleavage. Once F XII is activated, it activates prekallikrein to kallikrein that further activates F XII. Moreover, activated F XII initiates the intrinsic coagulation cascade by activating F XI. Therefore, at least three possibilities were considered to explain the defect of the abnormal F XII Bern: (1) an inability to bind to a negatively charged surface; (2) an impaired proteolytic cleavage by plasma kallikrein; and (3) a deficient proteolytic activity against its substrates, prekallikrein and/or F XI.

F XII Bern was adsorbed to kaolin as readily as normal F XII (Fig 4), ruling out a defective surface binding of F XII Bern. The proteolytic cleavage of F XII Bern by exogenous plasma kallikrein in the presence of dextran sulfate was comparable with the cleavage of normal F XII (Fig 5). Thus, the failure of F XII Bern to become enzymatically active is not due to its inability to be cleaved by plasma kallikrein. The slightly faster cleavage of normal F XII as compared with F XII Bern was probably due to the additional plasma kallikrein that was generated upon dextran sulfate activation of NHP. The kallikrein-induced heavy chains of both abnormal and normal F XII had similar size (Fig 5).

In the presence of dextran sulfate or kaolin, F XII Bern was cleaved by plasma kallikrein similarly to normal F XII. However, no complexes of α-F XIs with plasma protease inhibitors were observed in the proposita’s plasma in contrast to activated NHP, where high mol wt bands were shown by immunoblotting of F XII using unreduced SDS-PAGE (Fig 5). Upon contact activation of normal plasma, generated F XIIa is subsequently inactivated by protease inhibitors, mainly C1 inhibitor. Upon inactivation, a bimolecular enzyme-inhibitor complex is formed. This com-

![Fig 5. Cleavage of F XII by exogenous plasma kallikrein. Proposita’s plasma (F XII Bern) or F XII-deficient plasma containing 10% (vol/vol) NHP (NHP), was incubated with dextran sulfate (12.5 μg/mL) and exogenous plasma kallikrein (12 μg/mL) at 4°C. After various incubation intervals, aliquots of the reaction mixtures were removed and subjected to immunoblotting of F XII under nonreducing (upper panels) or reducing conditions (lower panels). Mol wts \( \times 10^{-3} \) (Kd) of prestained protein standards are indicated.](image)

![Fig 6. Cleavage of F XI and prekallikrein by activated F XII in plasma. F XII-deficient plasma containing 10% NHP (sample 1) and proposita’s plasma (sample 2) were incubated with kaolin in the presence (+) or absence (−) of exogenous plasma kallikrein. (A) After 2 hours of activation, the samples were reduced and subjected to immunoblotting of F XII. Analogous experiments were performed with the addition of traces of either radiolabeled F XI (B) or radiolabeled prekallikrein (C), and the X-ray films were directly exposed to the nitrocellulose membranes. Mol wts \( \times 10^{-3} \) (Kd) are indicated.](image)
plex formation requires the enzymatic active site of F XIIa. Our immunoprecipitation experiments with anti-C1-inhibitor antiserum and anti-α2-antiplasmin antiserum indicated that the α-F XIIa-inhibitor complexes with mol wts of about 195 and 145 Kd were due to α-F XIIa complexed with C1 inhibitor and α2-antiplasmin, respectively. This finding is in agreement with other reports. The fact that plasma kallikrein-cleaved F XII Bern is not able to form complexes with these protease inhibitors indicated that the defect in this variant molecule either affects the active site or interferes with the binding to protease inhibitors.

To further confirm the defective enzymatic function of F XII Bern, the proposita’s plasma was incubated with kaolin and plasma kallikrein in the presence of 125I-F XI or 125I-prekallikrein. Whereas both normal F XII and F XII Bern were fully cleaved by plasma kallikrein in the presence of kaolin (Fig 6A), partial proteolytic cleavage of 125I-F XI added as a tracer was only observed with NHP but not with proposita’s plasma (Fig 6B). Similarly, 125I-prekallikrein was fully cleaved in NHP activated by kaolin and plasma kallikrein, whereas only a trace amount of cleaved 125I-prekallikrein was observed with proposita’s plasma (Fig 6C).

Thus, the defect of F XII Bern affects the active site in the light chain region of this molecule and resembles the functional defect found in F XII Washington. Amino acid analysis of a tryptic peptide of the latter molecule indicated that the structural defect was due to a replacement of Cys-571 by Ser, thus disrupting the so-called serine disulfide loop near the active site serine. Further studies will be necessary to characterize the structural defect of F XII Bern.

ACKNOWLEDGMENT
The technical assistance of I. Sulzer is gratefully acknowledged.

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