Sulfatide-dependent Autoactivation of Human Blood Coagulation Factor XII (Hageman Factor)*

Guido Tans†, Jan Rosing‡, and John H. Griffin

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037 and the Department of Biochemistry, University of Limburg, Maastricht, The Netherlands

When purified human blood coagulation Factor XII (Hageman factor) is incubated with sulfatides at 37 °C, activation of Factor XII occurs as judged by the appearance of amidolytic activity towards the chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide. Polyacrylamide gel electrophoresis studies using 125I-Factor XII as a marker show that the appearance of amidolytic activity correlates with Factor XII cleavage, that activation goes to completion and that virtually all Factor XII, formed is present as the two-chain, 80,000 M, form, α-Factor XII. Rigorous analysis of kinetic data establishes that, between 0.02 and >90% of the reaction, the activation of Factor XII is described by a mechanism of autoactivation of Factor XII by Factor XII. The rate of autoactivation increases with increasing Factor XII concentrations at constant sulfatide levels but decreases with increasing sulfatide concentrations at constant levels of Factor XII. These findings suggest that the concentrations of Factor XII and Factor XII, bound to the sulfatide surface determine the rate of autoactivation. Soybean trypsin inhibitor, Trasylol, and anti-prekallikrein antibodies have no influence on the rate of sulfatide-dependent autoactivation of Factor XII. Benzamidine inhibits autoactivation with an inhibitor constant, K, of 1.9 mM which is similar to the K, of 1.6 mM for the enzyme, α-Factor XII. Thus, sulfatide-dependent activation of purified Factor XII is not due to contaminating proteases and is described by a second order mechanism of autoactivation due to the action of surface-bound Factor XII on surface-bound Factor XII.

Human blood coagulation Factor XII (Hageman factor) is the zymogen form of a serine protease that plays a central role in the early phase of intrinsic coagulation, kinin formation, and fibrinolysis (for recent reviews see Refs. 1 and 2). Upon exposure of human plasma to negatively charged surfaces such as glass or kaolin, these contact activation reactions are initiated. During contact activation, Factor XII and prekallikrein are thought to participate in a reciprocal activation reaction in which Factor XII, activates prekallikrein into kallikrein and, conversely, kallikrein activates Factor XII into Factor XIIa. A third protein, high molecular weight kininogen, functions as a nonenzymatic cofactor which enhances the rate of these reactions.

Factor XII has been suggested to be capable of activating its own zymogen, Factor XII, in the presence of glass, kaolin, or dextran sulfate (3-5), thus enhancing the rate at which Factor XII, will be formed during the early stages of contact activation. An explicit model in which Factor XII, activates Factor XII was presented by Silverberg et al. (5). However, their study was limited by the fact that only 5% of the available Factor XII became activated. No detailed kinetic analysis of the autoactivation of Factor XII has been reported.

Cerebroside sulfates (sulfatides) provide a very effective surface for the reactions taking place during contact activation (8, 9). Moreover, sulfatides are physicochemically homogeneous and provide an isotropic model surface on which to study protein-protein interactions responsible for contact activation reactions. The present report concerns a detailed kinetic analysis of the activation of purified Factor XII in the presence of sulfatides in an effort to establish the mechanism of this reaction. This analysis not only gives strong evidence for the process of autoactivation but also is a prerequisite to establishing quantitatively the relative importance of this process during contact activation of human plasma in the presence of sulfatides.

MATERIALS AND METHODS

All reagents used were of the highest grade commercially available. Chemicals used in the purification of Factor XII and kallikrein were obtained from sources used previously (10,11). 

Human blood coagulation Factor XII (HFF, Hageman Factor)* was produced in the purlification of Factor XII and kallikrein were obtained from sources used previously (10,11). 

Human Factor XII was purified using a modification of the original procedure (12) as described previously (11). Factor XII preparations were apparently homogeneous as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS), amino acid analysis, and analysis of the amino-terminal sequence. 

Factor XII, was purified as described previously (11). Anti-human prekallikrein was immunoprecipitated using affinity immobilized Factor XII-Sepharose. Adherent antibodies were eluted with 0.1 M NaCl, pH 7.4, and stored at -80°C.

Factor XIIa denoted here as α-Factor XIIa is sometimes mentioned as Factor XIIa (HFα) when the 28,000 M, form, β-Factor XIIa, is sometimes referred to as Factor XII, (HFF, Hageman factor fragment).

Materials and Methods are presented in miniature as printed by the authors. Miniature is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-3015, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

The abbreviations used are: DFP, diisopropylphosphofluoridate; SBTI, Soybean Trypsin Inhibitor; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, S 2302, H-D-Pro-Phe-Arg-p-nitroanilide; S 2222, N-benzoyl-L-Ile-L-Glu-L-Gly-Arg-p-nitroanilide.

8215
Surface-dependent Autoactivation of Coagulation Factor XII

1Labeled Factor XII was prepared using the chromatography method (12). The specific radioactivity was 37.6,000,000 cpm/μg. Protein concentrations were routinely determined according to Bradford (13) using bovine serum albumin as a standard. A 1/50 dilution of Factor XII and human fibrinogen standard was determined by amino acid analysis (14). Protein preparations were allowed to equilibrate at 4°C in 0.1% acetic acid, 500 mM NaCl, 10 mM Tris-HCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4. One standard assay system was prepared. The buffer solution was also adjusted to pH 7.4, and stored at -80°C until use.

Activation studies were carried out in solution 1, a 1:1 mixture of water and ethanol, to prevent loss of Factor XII to the glass surface. The tubes were preincubated for at least 16 hours in reaction buffer consisting of 50 mM NaCl, 0.5 mM MgCl₂, 100 mM Tris-HCl, pH 7.4. Before use, the tubes were washed with reaction buffer and dried before use. Control experiments showed this treatment was necessary because the initial rate of autoactivation was increased in the reaction tube. In a typical activation experiment, Factor XII was preincubated with 100 mM NaCl and 0.5 mM MgCl₂ at 37°C for 15 min in the presence of 0.5 μM Warfarin buffer pH 7.6, 50 μl distilled water, 50 μl 20% bovine serum albumin and 20 μl storage buffer. The assay system was then added with the addition of 200 μl of the sulfatide solution (prepared at 37°C) in the appropriate buffer. The final concentration of the activation mixture was 300 mM NaCl, 0.5 mM MgCl₂, 10 mM Tris-HCl, 0.5 mM EDTA, 0.01% SDS, 1 mg/ml ovalbumin and varying amounts of Factor XII and sulfatides (Fig. 1A). After 30 min, aliquots were removed and stored at -80°C until used.

RESULTS

To study surface-dependent activation of purified Factor XII, the amidolytic activity of Factor XII was used to determine activation and the coincidence of Factor XII cleavage and activation was established. Factor XII amidolytic activity was measured using either S 2302 or S 2222 as a chromogenic substrate. However, since the Km for S 2302 is considerably lower than the Km for S 2222 while the Km for both substrates is approximately the same (15), it was more economical to use S 2302. Fig. 1 shows that when purified Factor XII (18 μg/ml) was incubated with sulfatides (150 μM) at 37°C at pH 7.2, amidolytic activity was generated. The time course of amidolytic activity showed a typical sigmoidal curve. After an initial lag period of approximately 5 min, a rapid increase in amidolytic activity was observed and the appearance of amidolytic activity showed a typical sigmoidal curve.

The influence of pH on the rate of activation of Factor XII in the presence of sulfatides was determined as follows. Factor XII (18 μg/ml) was added to 200 μl of Factor XII activation mixture and incubated in the presence of sulfatides as described above. The final concentration of Factor XII in the activation mixture was 0.5 μM. The activation mixture was incubated at 37°C for 30 min in the presence of 0.5 μM Warfarin buffer pH 7.6, 50 μl distilled water, 20 μl serum albumin (20% v/v) and 20 μl 0.1 M NaCl and 0.5 mM MgCl₂. The change in optical density at 405 nm was monitored at a 20°C/min temperature change in directly coupled to an absorbance spectrophotometer. The experimental procedure was repeated three times.

Some of the experiments presented below were repeated using S 2222 as a substrate. Since the ratio of S 2222 to S 2302 was analyzed using a second order log-log plot, a straight line was obtained (Fig. 2B). A similar experiment was performed in which Factor XII was activated in the presence of 5 μg/ml of dextran sulfate.
of Factor XII in the presence of dextran sulfate appeared to follow the same autoactivation mechanism (Fig. 2) between 0.5 and 90% activation. A slight deviation from this mechanism was, however, found between 0.02% and 0.5% activation since the intercept at time 0 did not fall on the line. We were unable to reduce this deviation by addition of SBTI at 1 mg/ml (see also Table I and "Discussion").

The effect of variation of the initial amount of Factor XII, present in the activation mixture at time 0 is shown in Fig. 3. When the total concentration of Factor XII plus Factor XII, present is constant, the apparent second order rate constant as obtained from the slope of a second order logarithmic plot should remain constant. The intercept of such a plot is equal to \ln ([XIII]/[XII]) at time 0. Accordingly, when the initial amount of Factor XII, is varied while keeping the total amount of Factor XII plus Factor XII, constant, a set of parallel lines should be obtained. Variation in the initial amount of Factor XII, was achieved as follows: 14 μg/ml of Factor XII was activated in the presence of 25 μM sulfatides. After 0, 4, or 7 min, a small aliquot was added to a second activation mixture in which sulfatides and Factor XII were present in excess over Factor XII, The total amount of sulfatides present in the experiment was 150 μM and the total amount of Factor XII plus Factor XII, was kept constant at 28 μg/ml in all three experiments. Immediately after addition of the Factor XII, a sample was withdrawn and the amidolytic activity was measured. As seen in Fig. 3A, the presence of Factor XII, shortened the lag time considerably. However, the apparent second order rate constant of autoactivation derived from the slopes of the line in Fig. 3B was not affected. The initial amounts of Factor XII, present were 0.02, 0.25, and 1.56%, respectively, of the total Factor XII. Addition of β-Factor XII, the 28,000 M, form of Factor XII, that does not bind to negatively charged surfaces (19), up to amounts as high as 2 μg/ml (i.e. ~8% of the total Factor XII) did not influence the time course of activation (data not shown). Therefore, the presence of the surface binding site in the Factor XII, molecule is a prerequisite for the propagation of the reaction. Fig. 3 shows that the intercept at time 0 was equal to \ln ([Factor XII]/[Factor XII,]) measured at time 0, indicating that the mechanism described in Equations 2 and 3 adequately describes the reaction throughout the whole time course of activation. The surface-dependent activation of purified Factor XII between 0.02 and 90% of the reaction is, therefore, adequately described by a second order kinetic model representing autoactivation.

It was not possible to establish if the reaction from 90% up to 100% was fit according to this mechanism since the experimental error of ±2% in amidolytic assays precluded such an analysis once 90–95% activation was achieved.

The effect of varying concentrations of Factor XII and sulfatides on the rate of activation was studied. Fig. 4 shows the activation of varying amounts of Factor XII in the presence of 150 μM sulfatides. The maximal Factor XII, activity (Fig. 4A) was proportional to the amount of Factor XII present, indicating that the reaction went to completion in each case. Fig. 4B shows that the apparent k2 was a function of the amount of Factor XII present. When the amount of Factor XII was increased, the apparent k2 increased. Fig. 5 shows that the rate of autoactivation of Factor XII was also a function of the amount of sulfatides present. In this experiment, a constant amount of Factor XII (14 μg/ml) underwent activation in the presence of varying amounts of sulfatides. The apparent k2 increased with decreasing amounts of sulfatides present in the activation mixture.

To address the question of whether a contaminating zymogen form of a protease (e.g. prekallikrein) significantly contributed to this phenomenon of autoactivation, the effect of various inhibitors on the rate of Factor XII autoactivation in the presence of sulfatides was investigated. Table I shows that the apparent k2 of autoactivation for 14 μg/ml of Factor XII in the presence of 150 μM sulfatides was not affected by the presence of 0.5 mg/ml of SBTI, 300 units/ml of Trasylol, or 100 μg/ml of immunopurified antibody against human prekallikrein. These data support the hypothesis that the activation of Factor XII is due to Factor XII, since these inhibitors do not inhibit Factor XII, but do inhibit kallikrein, plasmin, and many other plasma proteases. These data also argue against a possible contamination of our Factor XII preparation with prekallikrein. Benzamidine is a competitive inhibitor of both Factor XII, and kallikrein as well as other proteases; however, the K, values differ for different enzymes. Dixon plots (20) for the inhibition of kallikrein and Factor XII...
FIG. 2. Activation of Factor XII (14 μg/ml) in the presence of 150 μM sulfatides or 5 μg/ml of dextran sulfate. A, Factor XII (5.6 μg) was preincubated at 37°C for 5 min in a total volume of 200 μl of reaction buffer. Reaction was started by the addition of 200 μl of reaction buffer containing sulfatides (○) or dextran sulfate (●). Final concentrations were 14 μg/ml of Factor XII and 150 μM sulfatides or 5 μg/ml of dextran sulfate. At various times, samples were withdrawn and assayed for Factor XII. B, from the observed ampidolytic activities, the fractions of Factor XII formed and of Factor XII remaining were calculated, assuming all available Factor XII had been activated when the maximal value of ampidolytic activity had been reached. From this, the value of In ([Factor XII]/[Factor XII]) was calculated for each time point. For further experimental details see “Materials and Methods.”

FIG. 3. Effect of varying the initial amount of Factor XII, on the sulfatide-dependent activation of Factor XII. Variation of the initial amount of Factor XII present was achieved by preincubation of 21 μg/ml of Factor XII with 25 μM sulfatides for 0 (○), 4 (●), or 7 min (▲). 10 μl of this preincubation mixture was then added simultaneously with 200 μl of sulfatides in reaction buffer to 190 μl of reaction buffer containing 11 μg of Factor XII that had been preincubated at 37°C for 5 min. The total Factor XII and sulfatide concentrations in this activation mixture were 25 μg/ml and 150 μM, respectively. At various times, samples were withdrawn and assayed for Factor XII. The ratio of [Factor XII]/[Factor XII] was calculated and the second order plot (Equation 3) was made (B).

XII, under appropriate experimental conditions are shown in Fig. 6. From these plots, the $K_i$ values of benzamidine for kallikrein and Factor XII, were determined to be 0.27 and 1.5 mM, respectively. The $K_i$ of inhibition of autoactivation by benzamidine was determined. Fig. 7A shows the effect of benzamidine on sulfatide-dependent autoactivation of Factor XII. The apparent $k_2$ decreased with increasing benzamidine concentrations. Fig. 7B shows the apparent $k_2$ determined from these plots at the different benzamidine concentrations used. These points were fit well by a simulated curve (Equation 6 under “Materials and Methods”) assuming competitive inhibition by benzamidine with a $K_i$ of 1.9 mM. As a comparison, the simulated curves for $K_i = 1.5$ and 0.27 mM are shown. These data virtually exclude the possibility that kallikrein was responsible for the activation of Factor XII in the presence of sulfatides.

The effect of pH on the rate of sulfatide-dependent autoactivation of Factor XII was investigated. Fig. 8 shows that the apparent $k_2$ was markedly affected by small changes in pH. A sharp optimum was observed at pH 7.4. The results shown in Fig. 8 indicate that the pH has to be controlled very carefully since slight changes in pH can have a dramatic effect on the rate of surface-dependent autoactivation of Factor XII.

DISCUSSION

The experiments presented in this paper show that purified human Factor XII is rapidly activated upon incubation with sulfatides or dextran sulfate. Our data confirm and extend earlier reports in which activation of Factor XII occurred in the presence of glass, kaolin, or dextran sulfate (3–7). At least four mechanisms can be put forward to explain this phenomenon. First, a contaminating protease may activate surface-bound Factor XII. Second, Factor XII may be capable of activating Factor XII in a reaction stimulated by the presence of the negatively charged surface. Third, Factor XII preparations might contain trace amounts of a contaminating zymogen (e.g. prekallikrein) that is activated in the presence of the negatively charged surface either by trace amounts of Factor XII or by other mechanisms. The activated contaminant may then be responsible for the ensuing rapid activation of surface-bound Factor XII. Fourth, upon binding to the surface, the Factor XII zymogen could activate itself (intramolecular) or
other Factor XII molecules (intermolecular). For example, this is the case for the activation of pepsinogen to pepsin (21, 22).

Since the time course of sulfatide-dependent Factor XII activation is sigmoidal in time at all the different Factor XII and sulfatide concentrations studied, the presence of a contaminating protease as a significant contribution to the activation of Factor XII can be ruled out. This was pointed out earlier by Silverberg et al. (5) for the activation of Factor XII occurring in a quartz cuvette. Although the other three mechanisms mentioned do not exclude each other and may contribute simultaneously to various extents, the reaction surface-dependent activation of Factor XII by Factor XII, can account for the observed phenomena. The data presented are consistent with the activation of Factor XII in the presence of sulfatides according to the second order reaction mechanism:

\[
\text{Factor XII} + \text{Factor XII} \xrightarrow{k_2} 2 \text{Factor XII}
\]

This mechanism is described as surface-dependent autoactivation of Factor XII. A rigorous analysis of the time course of Factor XII activation indicates this mechanism of autoactivation accounts for 0.02 to 90% of the reaction over the studied range of Factor XII concentrations (3.5 to 30 μg/ml) and sulfatide concentrations (25 to 150 μM). No deviation from this mechanism at high Factor XII concentrations was observed. This indicates that these concentrations are still well below the \( K_a \) for this reaction.

The data presented in Fig. 5 show that decreasing the sulfatide concentration results in an increase in the rate of autoactivation and, therefore, the observed \( k_a \) should be regarded as an apparent \( k_a \) This can be explained in a model in which the rate of autoactivation is determined by the concentrations of substrate Factor XII, and enzyme Factor XII, bound to the sulfatide surface. In this case, both an increase in Factor XII at a constant sulfatide concentration or a decrease in sulfatides at a constant Factor XII concentration will result in an increase of the local concentrations of Factor XII and Factor XII, and will result in an increase of the rate of autoactivation (\( k_{\text{app}} \)). Such a hypothesis is supported by the fact that addition of 2 μg/ml of β-Factor XII did not alter the time course of activation of Factor XII (14 μg/ml) in the presence of 150 μM sulfatides. β-Factor XII, which is derived from α-Factor XII, through proteolytic cleavage (19, 23, 24), lacks the binding site for negatively charged surfaces (19, 24). This is in agreement with the data of Silverberg et al. (5) who showed that β-Factor XII, did not support activation of Factor XII in a quartz cuvette. In contrast to β-Factor XII, addition of α-Factor XII (Fig. 3) does indeed affect the time course of activation by shortening...
the lag period. Therefore, the presence of a Factor XII-binding site on the sulfatide surface is a prerequisite for the propagation of the reaction. We conclude that surface-bound α-Factor XII is the enzyme responsible for cleaving and activating surface-bound Factor XII.

This kind of mechanism and the data that support it are reminiscent of the situation for the effect of phospholipids on the $K_m$ for prothrombin and Factor X activation described earlier (25, 26). However, no binding data for the binding of Factor XII to sulfatides are as yet available to establish the exact relationship between the apparent $k_0$ and Factor XII concentrations at the sulfatide surface.

The evidence presented thus far does not rule out the possibility of a contaminating zymogen that becomes activated by Factor XII, in the presence of a negatively charged surface with the consequence that this activated zymogen then in fact represents the actual enzyme principally responsible for activating Factor XII. In order for a mechanism involving a contaminating zymogen to be important, the activated zymogen should be a potent activator of Factor XII and, conversely, it should be effectively activated by Factor XII. Plasma prekallikrein is known to meet these criteria. Hence, experiments to address this possibility were conducted. The data presented here, however, virtually exclude the possibility that prekallikrein is present in our Factor XII preparations since potent inhibitors of kallikrein such as soybean trypsin inhibitor, Trasylol, or anti-prekallikrein antibodies have no effect on the sulfatide-dependent activation of Factor XII. Moreover, benzamidine does inhibit autoactivation but the inhibition constant, $K_i$, measured (1.9 mM) is much higher than the $K_i$ of benzamidine for kallikrein (0.27 mM). The fact that the $K_i$ of benzamidine on the process of autoactivation is very close to the $K_i$ measured for α-Factor XII, i.e. 1.9 mM as compared to 1.5 mM, makes it unlikely that a contaminating zymogen such as prekallikrein is responsible for the observed activation of Factor XII. Finally, the essential argument that excludes a significant role for a contaminating zymogen such as prekallikrein is based on the fact that the activation of Factor XII in the presence of a Factor XII-dependent surface is kinetically modeled by a mechanism of autoactivation over the whole time period of activation from 0.02 to 90% of the reaction at all Factor XII and sulfatide concentrations tested. If the presence of a contaminating zymogen were solely responsible for the observed lag time and subsequent rapid activation of Factor XII, a change in the order of the reaction would occur and deviations on the second order plot would become evident once this zymogen were fully activated. However, the data presented here show clearly that this is not the case.

The results presented in this paper are all consistent with the autoactivation mechanism presented above. However, this does not mean that other mechanisms might not contribute when other surfaces or other Factor XII preparations are analyzed under different conditions. Purified bovine Factor XII has been reported not to exhibit autoactivation in the presence of quartz or kaolin (27). In our studies, other possible mechanisms, if they do occur, do not significantly contribute to the activation of Factor XII over the range of activation studied from 0.02 to 90%. Nonetheless, during the course of our studies some Factor XII preparations showed slight deviations from linearity in second order plots in the range between 0.02 and 0.5% activation in the presence of sulfatides. For the rest of the range up to 95% activation, the second order plot was linear and the apparent $k_0$ was the same for all different preparations of Factor XII tested. The presence of soybean trypsin inhibitor substantially reduced this deviation, indicating that it was likely that these preparations may have had traces of a protease present.

Considerable attention has been given to the question of how the negatively charged surface triggers the contact activation reactions in human plasma. It has been suggested that binding of Factor XII to the negatively charged surface results in a conformational change of the Factor XII zymogen, rendering it much more susceptible to proteolytic activation (28). Since it has been shown that the zymogen, prekallikrein, can incorporate DFP at a slow rate, it is possible that it is either kallikrein or prekallikrein acting on the surface-bound Factor XII during the initial stage (29). In this way, the reciprocal activation of Factor XII and prekallikrein is initiated. Others have suggested that autoactivation of Factor XII by Factor XII...
constant, of autoactivation of Factor XI in the presence of sulfatides.

The final concentrations reached were 14 μg/ml of Factor XII and 150 μM sulfatides, and no benzamidine (○), 0.27 (△), 0.75 (□), 1.3 (■), 3 (△), or 5 mM (●) benzamidine, respectively. At various times, samples were withdrawn and assayed for Factor XII, and, from the observed ΔAαα/min, ln ([Factor XII]/[Factor XII]) was calculated. From the second order plots shown in A, the apparent k2 was determined. B shows the apparent k2 obtained as a function of the benzamidine concentration. The lines shown are simulations based on Equation 6 assuming competitive inhibition with a KI of, respectively, 1.9 mM (solid line), 1.5 mM (upper dashed line), or 0.27 mM (lower dashed line).

**Acknowledgments**—We gratefully thank Craig Berridge for skillfully purifying Factor XII and Elizabeth S. Simpson for excellent secretarial assistance.

**REFERENCES**

Surface-dependent Autoactivation of Coagulation Factor XII

Sulfatide-dependent autoactivation of human blood coagulation Factor XII (Hageman Factor).

G Tans, J Rosing and J H Griffin


Access the most updated version of this article at http://www.jbc.org/content/258/13/8215

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/13/8215.full.html#ref-list-1

Downloaded from http://www.jbc.org/ by guest on August 29, 2017