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AUTOACTIVATABILITY OF HUMAN HAGEMAN FACTOR (FACTOR XII)

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SUMMARY

Purified Hageman factor was found to autodigest upon binding to a negatively charged surface such as kaolin. Assessment by incorporation of tritiated diisopropylfluorophosphate indicated that this cleavage was accompanied by activation and that the two known forms of activated Hageman factor result. Cleavage within a critical disulfide bridge generated activated Hageman factor, a two-chain enzyme of molecular weight 80,000 as well as the active Hageman factor fragment, a 28,000 molecular weight cleavage product. The autocleavage seen was dependent upon the percentage of activated Hageman factor in the starting material and was independent of HMW-kininogen. This result suggests that initiation of the intrinsic coagulation cascade may, in part, depend upon the autoactivatability of Hageman factor described herein. This observation may in turn, account for the ability of prekallikrein deficient plasma to gradually autoactivate as a function of the time of contact with initiating surfaces.

INTRODUCTION

Initiation of the intrinsic coagulation pathway requires the presence of at least three plasma proteins; namely, Hageman factor (factor XII),† HMW-kininogen, and factor XI. When bound to certain negatively charged surfaces, the factor XII is activated and it in turn activates factor XI to factor XIa (1). This step is dependent upon 1:1 molar binding of HMW-kininogen and factor XI (2) and attachment of the complex to the surface (3). Prekallikrein functions as an accelerator of this reaction sequence (4) via enzymatic activation of Hageman factor by kallikrein (5), but prekallikrein is not an absolute requirement for initiation. Thus, pre-

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† Abbreviations and synonyms used: Hageman factor (Factor XII), activated Hageman factor (HFa or factor XIIa), active Hageman factor fragment (HFf or factor XIIf), diisopropyl fluorophosphate (DFP), high molecular weight kininogen (HMW-kininogwn), .01M phosphate buffer. 15M Nacl pH 7.8 (PBS), molecular weight (mol.wt.) and sodium dodecyl sulfate (SDS).

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kallikrein deficient plasma autoactivates as the time of contact with a surface is increased (6).

The mechanism by which the first active site is generated to initiate the cascase is unclear. Ratnoff and Saito have presented evidence to suggest that binding of factor XII to a surface exposes an active site in the absence of cleavage as assessed by digestion of a synthetic substrate (7). However, studies of the incorporation of ^{3}H-DFP into DFP-treated factor XII (so as to inactivate as much activated factor XII as possible) failed to demonstrate a significant increase in active site formation upon binding to kaolin (8). Wiggins et al have demonstrated that rabbit activated factor XII could activate native rabbit factor XII and suggested an autoactivation mechanism (9) however, kinetics consistent with autoactivation were not reported. We have examined the ability of highly purified human factor XII to autoactivate upon binding to kaolin. Our result suggests that autocleavage can occur in man in which both factor XIIa (or HFa), the activated enzyme resulting from cleavage within a disulfide bond, is formed (10), as well as factor XIIf (or HFf) the active fragment (11) formed by scission of the molecule into two major pieces (12,13). The rate of cleavage was proportional to the quantity of HFa contaminating the factor XII preparation and active site formation was demonstrable as assessed by incorporation of ^{3}H-DFP into the cleavage products.

METHODS

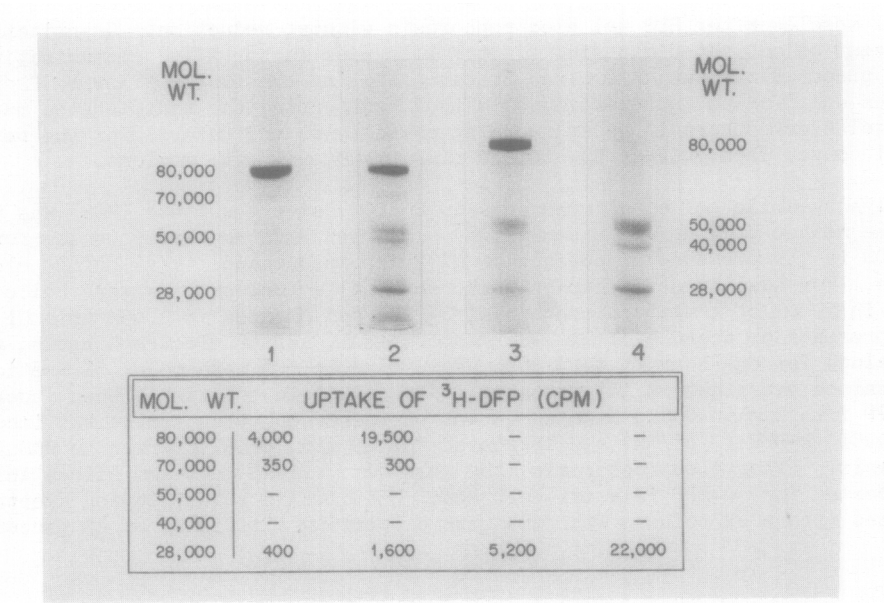
Factor XII was purified by a modification of the method described by Griffin and Cochrane (14). Some preparations utilized 10mM benzamidine and 10^{-4} DFP in all buffer systems to minimize the formation of activated factor XII, while other preparations eliminated either the DFP or both the benzamidine and the DFP. In addition, a final pH gradient on SP Sephadex utilizing Gomori's tris maleate buffer was added to the procedure to remove trace contaminants (starting buffer, tris maleate pH 5.8, conductivity 5 mS; limit buffer, tris maleate pH 8.4, conductivity 12 mS). When assessed at a concentration of 1.0 mg/ml, the resulting factor XII preparations contained less than 0.01 percent contamination with HMW-kininogen, factor XI, prekallikrein, or plasminogen, which represents the limit of our ability to detect them. The preparations contained varying amounts of activated Hageman factor as assessed by SDS gel electrophoresis. The uncleaved zymogen had a molecular weight of 80,000 whether reduced or not; factor XIIa was found at a molecular weight (mol.wt.) of 80,000 when not reduced but yielded a heavy chain of 50,000 and a light chain of 30,000 when assessed under reducing conditions. Cleavage to factor XIIf was indicated by the appearance of similar 50,000 and 30,000

mol. wt. species after SDS gel electrophoresis without reduction. Quantitation of the percent of activated material (factor XIIa plus factor XIIf) contaminating factor XII preparations was determined by radiolabeling the factor XII with ^{125}Iodine using the solid-phase lactoperoxidase method, subjecting the radiolabeled material to SDS gel electrophoresis under reducing conditions, and determining the percentage of total counts recovered at the 50,000 plus 30,000 mol. wt. regions.

Polyacrylamide gel electrophoresis in Sodium dodecyl sulfate (SDS) was performed using the method of King and Laemmli (15). Samples were routinely boiled for 2 min in 4% SDS in the presence or absence of 1% 2-mercaptoethanol (v/v) before electrophoresis. For assessment of kaolin-bound Hageman factor, samples were boiled for two minutes in 20 ul of a mixture containing 3% SDS and 8M urea in 0.05M tris Cl pH 6.8 in the presence or absence of 1% 2-mercaptoethanol. After electrophoresis, gels were stained for one hour in 0.2% Coomassie Blue in 50% methanol-7.5% acetic acid and destained overnight in 15% methanol 10% acetic acid solution. When incorporation of ^{3}H-DFP into kaolin-bound Hageman factor was assessed, each sample was incubated with 50 uCi ^{3}H-DFP (3 ci/mM) and incubated for 30 min. at 37°C prior to SDS gel electrophoresis. After electrophoresis, the gels were sliced into 1mm slices and each slice counted for ^{3}H-DFP in a tri-carb Model 3375 liquid scintillation spectrometer calibrated with a ^{3}H toluene standard with a counting efficiency of 45 percent.

RESULTS

Replicate samples containing 10ug Hageman factor of which 25% was activated were either incubated in 20ul .01M phosphate buffer .15M NaCl pH 7.8 (PBS) for 10 min at 37°C or incubated with 20ul kaolin suspension (1.0 mg/ml in PBS) and incubated for 10 min at 37°C. Each sample was then assessed by SDS gel electrophoresis with or without reduction with 2 mercaptoethanol. The gels were stained with Coomassie blue and are shown in Fig. 1. Positions 1 and 3 represent nonreduced and reduced Hageman factor, respectively, without surface contact, while positions 2 and 4 represent non-reduced and reduced Hageman factor after elution from kaolin. The experiment was then repeated, except each sample was treated with ^{3}H-DFP and further incubated for 30 min at 37°C prior to electrophoresis. The gels were then sliced and counted for tritium. The results for each condition are shown beneath the corresponding stained gel. Non-reduced Hageman factor without surface contact (position one), revealed a major band at mol. wt. 80,000 and trace bands at 50,000 and 30,000 representing a small amount of factor XIIf formation. When incorporation of ^{3}H-DFP was examined, 4,000 cpm were incorporated at mol. wt. 80,000 representing the factor XIIa in the preparation, 350 cpm were incorporated at mol. wt. 70,000,a minor factor XIIa cleavage product, while 400 cpm were found at mol. wt. 30,000 representing factor XIIf. Upon reduction (position 3) additional



MOL. WT.	UPTAKE OF ^{3}H-DFP (CPM)			
80,000	4,000	19,500	–	–
70,000	350	300	–	–
50,000	–	–	–	–
40,000	–	–	–	–
28,000	400	1,600	5,200	22,000

Figure 1 SDS polyacrylamide gel (9.0%) electrophoresis of purified human factor XII before and after incubation with kaolin for 10 minutes at 37°C. Positions 1 and 3 represent non-reduced and reduced Hageman factor, respectively without surface contact, while positions 2 and 4 represent non-reduced and reduced Hageman factor after elution from kaolin. The molecular weights of bands seen in non-reduced gels are shown at the left and in reduced gels are shown at the right. Below is shown the incorporation of ^{3}H-DFP into Hageman factor before (gels 1 and 3) and after (gels 2 and 4) incubation with kaolin. The counts/min. incorporated into each molecular weight species are shown below the corresponding stained gel.

breakdown to 50,000 and 30,000 mol. wt. bands is seen confirming the presence of factor XIIa in the starting material, and all of the counts (5,200) were found at mol. wt. 28,000. The 80,000 mol. wt. band remaining represents native factor XII which did not incorporate ^{3}H-DFP. It should be noted that, although the total incubation time was 40 minutes, examination of ^{125}I-factor XII before and after a 40-minute incubation revealed the same percent cleavage, thus further breakdown did not occur as a result of incubation in the absence of a surface. Non-reduced Hageman factor eluted from kaolin (position 2) revealed an increase in 50,000 and 30,000 mol. wt. bands compared to the non-reduced starting material (position one) indicating formation of factor XIIf. The ^{3}H-DFP uptake under these conditions, however, demonstrated formation of factor XIIa as indicated by incorporation of 19,500 cpm at mol. wt. 80,000 as well as factor XIIf as indicated by incorporation of 1,600 cpm

at a mol. wt. of 28,000. When reduced (position four), virtually complete depletion of the native 80,000 mol. wt. factor XII was seen, and in its place was a 50,000 mol. wt. heavy chain (seen as a doublet), a 40,000 mol. wt. heavy chain degradation product, and a light chain at mol. wt. 28,000. Under these conditions, all of the ^{3}H-DFP was incorporated into the 28,000 mol. wt. light chain.

This result suggested that the contaminating factor XIIa and/or factor XIIf in the factor XII preparation might be responsible for the cleavage and further activation seen. Therefore, preparations of factor XII at 0.5 mg/ml containing varying proportions of cleaved material were radiolabeled with ^{125}Iodine, and a time course of cleavage of each preparation upon binding to kaolin was performed.

The percentage factor XIIa and factor XIIf present in Hageman factor preparations was determined by subjecting ^{125}I-Hageman factor (1.2uCi/ug) to SDS gel electrophoresis under reducing conditions. The gels were sliced into 1.0mm segments and each counted for ^{125}Iodine. The percentage of ^{125}Iodine at 50,000 daltons plus 30,000 daltons was divided by the sum of the percentage of counts at 80,000, 50,000 and 30,000 to yield the percent cleavage. Samples of Hageman factor containing 2 x 10^{5} counts/min (2 ul of a 0.1 mg/ml solution) were incubated with 30 ul kaolin (1.0 mg/ml in 30 ul phosphate buffered saline containing .1% bovine serum albumin) for either 2, 5 or 10 minutes. 30 ul of a mixture containing 3% SDS and 8M urea in .05M tris Cl pH 6.8 were added, the samples made 1% 2-mercaptoethanol (v/v), and the mixture boiled for two minutes and subjected to SDS gel electrophoresis. The gels were sliced in 1.0mm slices, each slice was counted for ^{125}Iodine, and the per cent cleavage determined. Over 90% recovery of the applied counts was obtained.

As shown in Table I, the rate of cleavage was proportional to the percent of activated material contaminating each preparation. A preparation containing as little as six percent cleaved material was 60% activated by 10 minutes, while an 11% activated preparation was fully activated by 10 minutes, and a 22% activated preparation was fully activated by five minutes. When the six percent activated factor XII preparation was exposed to 10^{-2}M DFP for one hour at 37°C, dialyzed extensively in PBS, and then tested, only 18% cleavage was seen by 10 minutes.

TABLE 1

RELATIONSHIP OF AUTOCLEAVAGE OF HUMAN FACTOR XII TO THE PERCENTAGE OF ACTIVE ENZYME PRESENT IN THE STARTING MATERIAL

Time of Incubation with Kaolin	0 Min.*	2 Min.	5 Min.	10 Min.
Percent Cleavage	6	11	25	60
	11	30	70	95
	22	50	95	95

* Starting Material - Percent-factor XIIa plus factor XIIf

DISCUSSION

We therefore, conclude that human activated factor XII can digest and activate native, uncleaved factor XII when it is bound to initiating surfaces. The data shown in Fig. 1 are consistent with autocleavage and active site formation. The increasing rate of cleavage dependent upon the concentration of activated HF seen in Table I suggests a true autoactivation mechanism. Other studies (9) have shown HF autocleavage and active site formation as assessed by factor XI activation in a rabbit system, but linear rates of autocleavage were obtained which are not consistent with an autoactivation mechanism. We have recently demonstrated autoactivation kinetics of HF by traces of HFa (0.2%) utilizing a synthetic substrate; accelerated rate of HFa formation was seen and competition between native HF and the synthetic substrate for interaction with HFa was demonstrable (17).

The mechanism by which any factor XIIa might first form in plasma or upon binding to surfaces, however, remains unclear. The activation observed in purified surface-bound factor XII (7) (which was not exposed to inhibitors) may be explicable by the autoactivatability demonstrated above, even though obvious cleavage of the starting material was not evident; only a small fraction of a percent of factor XIIa would be needed. Although incubation of Hageman factor preparations with inhibitors such as DFP, markedly diminishes this rate of autoactivation, we cannot yet distinguish cleavage by traces of residual activated material from any intrinsic activity the zymogen might possess. Assuming the former possibility, a dia-

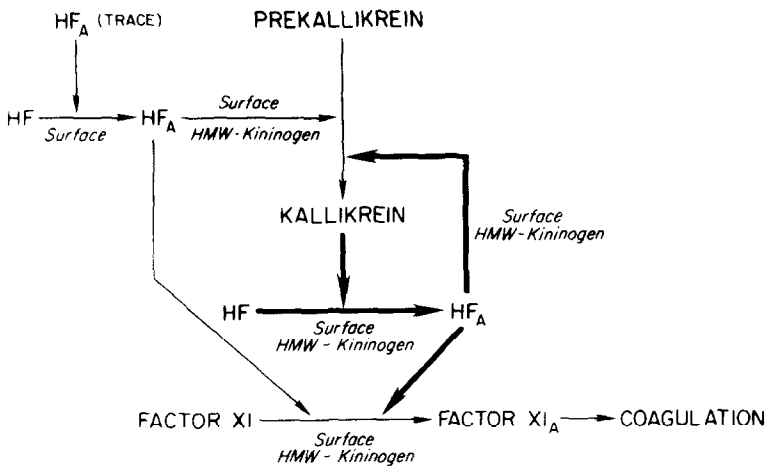


Figure 2 Schematic diagram of the initiating steps of the intrinsic coagulation pathway. The pathway shown by the thin lines on the left would account for factor XI activation in prekallikrein deficient plasma while the pathways shown by the thickened arrows are the major route by which activation occurs in normal plasma.

gram depicting the initiating events in contact activation is shown in Fig. 2. Traces of factor XIIa (HFa) initiate surface dependent Hageman factor activation by autocleavage, a reaction which is independent of HMW-kininogen. HFa, in turn activates prekallikrein and factor XI and this step is HMW-kininogen dependent. Kallikrein can then reciprocally activate surface bound factor XII, a reaction which is more rapid (shown by the thickened lines and arrows) so that this repre sents the major mode of activation in normal plasma within the first 1-2 minutes incubation with kaolin (4). In prekallikrein deficient plasma, a slower rate of activation is seen which autocorrects over time (6,16). This may depend upon the factor XII autoactivatability described herein followed by activation of the factor XI-HMW-kininogen complex (2).

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