Proc. Natl. Acad. Sci. USA
Vol. 75, No. 4, pp. 1998–2002, April 1978
Medical Sciences

Role of surface in surface-dependent activation of Hageman factor (blood coagulation Factor XII)

(kallikrein/kininogen/plasmin/Factor XI)

JOHN H. GRIFFIN

Department of Immunopathology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037

Communicated by Hans J. Müller-Eberhard, January 26, 1978

ABSTRACT **The mechanism by which negatively charged substances such as celite, kaolin, or ellagic acid contribute to the surface-dependent activation of Hageman factor (Factor XII) was studied. Kinetic studies of the proteolytic activation of ^{125}I-labeled human Hageman factor by human plasma kallikrein, plasmin, activated Factor XI, and trypsin were performed in the presence and absence of high molecular weight kininogen and surface materials such as celite, kaolin, or ellagic acid. The results showed that surface-bound Hageman factor was 500 times more susceptible than soluble Hageman factor to proteolytic activation by kallikrein in the presence of high molecular weight kininogen. Surface binding of Hageman factor enhanced its cleavage by plasmin, activated Factor XI, and trypsin by 100-fold, 30-fold, and 5-fold, respectively. On a molar basis, trypsin was twice as potent as kallikrein in the cleavage of the surface-bound Hageman factor, while plasmin and activated Factor XI were an order of magnitude less potent than kallikrein. Kallikrein even at concentrations as low as 0.5 nM (i.e., 1/1000th of the concentration of prekallikrein in plasma) was very potent in the limited proteolysis of the surface-bound Hageman factor. These results suggest that substances classically known as "activating surfaces" promote the activation of Hageman factor indirectly by altering its structure such that it is much more susceptible to proteolytic activation by other plasma or cellular proteases.**

Exposure of human plasma to glass, kaolin, celite, or other negatively charged surfaces initiates contact activation reactions that trigger the intrinsic coagulation pathway (1, 2), the kinin-forming pathway (3), and the fibrinolytic pathway (4, 5). Proteins that are known to be involved in contact activation of human plasma include Hageman factor (HF, blood coagulation Factor XII) (6), plasma prekallikrein (Fletcher factor) (7, 8), coagulation Factor XI (plasma thromboplastin antecedent) (9), and high molecular weight (M_r) kininogen, which was recognized in studies of Flaujeac trait (10), Fitzgerald trait (11, 12), Williams trait (13), and "contact activation cofactor" (14).

For many years it has been assumed, but never directly proven, that HF is directly activated by absorption to negatively charged surfaces (15). However, in the initial step of contact activation, recent studies using highly purified HF and [^{3}H]-diisopropyl phosphorofluoridate as a quantitative active site titrant established that surface-binding *per se* does not result in enhanced uptake of the active site titrant by human (16, 17) or bovine (18, 19) HF in the absence of other proteins.

These data, together with the observations that surface-bound HF is rapidly cleaved in plasma during contact activation in glass tubes (20) and that HF in solution can be proteolytically activated by plasmin, kallikrein, Factor XI_a, or trypsin (21–24), stimulated the present quantitative studies of the cleavage of purified human HF by these proteases in the presence and absence of various surface materials and high-M_r kininogen. In this report the limited proteolysis of ^{125}I-labeled HF (^{125}I-HF) was assessed using sodium dodecyl sulfate ($NaDodSO_4$) gel analysis to follow the cleavage of the 80,000 M_r single chain HF zymogen to give fragments of approximately 52,000 and 28,000 M_r.

MATERIALS AND METHODS

Hageman factor was isolated from freshly collected normal human plasma as previously described (25) and gave a single stained protein band on polyacrylamide gels run in the presence of $NaDodSO_4$ (26). The purified protein had a specific clotting activity of 80 units per mg protein, with 1 clotting unit defined as the amount of activity present in 1 ml of citrated normal human plasma. HF was radiolabeled with ^{125}I using the chloramine-T method (27). The ^{125}I-HF, containing from 2 to 8 μCi/μg in different preparations, retained its procoagulant activity, assayed as previously described (25).

Prekallikrein [also known as plasminogen proactivator (28)], Factor XI, and high-M_r kininogen were isolated from fresh human plasma by reported methods (29–31). The specific clotting activities of these three proteins were 19, 220, and 12 clotting units per mg, respectively, as assayed in the appropriate deficient plasmas. Each protein appeared to be greater than 95% homogeneous on $NaDodSO_4$ gels in the presence of reducing agent.

Kallikrein was prepared by activating the purified prekallikrein with purified activated HF in its 28,000-M_r form followed by separation of the kallikrein from the activated HF by DEAE-Sephadex chromatography.* The kallikrein generated in this way retained its full coagulant activity in correcting the coagulation defect of prekallikrein-deficient (Fletcher trait) plasma and possessed full activity in the hydrolysis of the tripeptide subtrate Bz-Pro-Phe-Arg-paranitroanilide.* Factor XI_a was generated from purified Factor XI (30) using the method outlined for the activation of prekallikrein. The Factor XI_a generated in this way retains full procoagulant activity as assayed in Factor XI_a clotting assays. In calculating the molar concentration of each protease, a M_r value of 85,000 was used for prekallikrein, (29), and 83,000 M_r was used for Factor XI_a (30). In the latter case it was assumed there are two active sites per Factor XI_a molecule of 160,000 M_r.

Plasminogen was isolated from fresh human plasma, and plasmin was generated by passage of the plasminogen over urokinase-agarose beads as previously reported (28). The molar concentration of active plasmin was quantitated using both

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: HF, Hageman factor; XI_a, activated Factor XI; M_r, molecular weight; $NaDodSO_4$, sodium dodecyl sulfate.

* B. N. Bouma and J. H. Griffin, unpublished data.

titration of the active site with paranitrophenylguanidinobenzoate and caseinolytic assays (28).

Trypsin was obtained from Worthington Biochemicals. Fresh stock solutions of trypsin were prepared before each experiment that used this protease by dissolving the lyophilized protein in a solution of 1 mM acetic acid to give a concentration of 1 mg/ml.

For studies of the cleavage of HF, reaction mixtures were incubated for various times at 37° in 1.4-ml conical polypropylene snap-cap centrifuge tubes. The order of addition of components to the tubes was as follows: 0.1 M TrisCl (pH 7.4), celite (or kaolin or ellagic acid), HF, and high-M_r kininogen. At this point each tube was agitated on a Vortex mixer and allowed to stand for 2 min at 20° to allow maximal binding of the proteins to the added surface. It was verified that the amount of surface material was sufficient to bind all of the HF and high-M_r kininogen. Then bovine serum albumin (Sigma Chemical) was added and the tube was agitated again and left at 20° for 2 min to allow saturation of remaining surface binding sites before the final addition of protease. Stock solutions of plasmin, kallikrein, and Factor XI_a were stored at −20° or −70° in storage buffer (5 mM Na acetate/0.15 M NaCl/0.5 mM EDTA/0.02% NaN_3, pH 5.0) at respective concentrations of 5 μM, 1.5 μM, and 0.30 μM. Each protease was diluted with Tris-buffered saline (0.01 M TrisCl/0.15 M NaCl/0.02% NaN_3, pH 7.4) containing bovine serum albumin at 1 mg/ml whenever necessary to achieve very low concentrations of protease prior to addition to the reaction mixtures. HF and high-M_r kininogen were stored at 4° in storage buffer.

The cleavage of ^{125}I-HF in each reaction mixture was assessed using $NaDodSO_4$ gel analysis in the presence of 2-mercaptoethanol (20, 21, 32). On reduced $NaDodSO_4$ gels, the ^{125}I-HF gave only three peaks, which corresponded to apparent molecular weights of 80,000, 52,000, and 28,000. The percent of HF cleaved was calculated using the integrated radioactivity for each peak and the observation that the disappearance of the 80,000-M_r peak was correlated with the appearance of the 52,000- and 28,000-M_r peaks.

RESULTS

Cleavage of Hageman Factor by Kallikrein. The limited proteolytic cleavage of human HF by different proteases was studied by following the cleavage of the ^{125}I-HF zymogen at 80,000 M_r to give fragments near 52,000 and 28,000 M_r as assessed using $NaDodSO_4$ gel analysis (20, 21, 32). The cleavage of ^{125}I-HF by kallikrein was linear with time during the cleavage of greater than 50% of the HF molecules (32). The initial rate of cleavage of HF was directly proportional to the concentration of kallikrein. In Fig. 1, the concentration of kallikrein was varied and the percent of HF molecules cleaved during a 9-min period was determined. The effects of the presence or absence of celite and of high-M_r kininogen on this reaction are seen in Fig. 1. Note that the lower abscissa records the concentration of kallikrein in the absence of celite while the upper abscissa, whose scale differs by 50, reflects the concentration in the presence of celite. A comparison of the initial slopes of the curves in Fig. 1 indicates that, in the absence of high-M_r kininogen, HF bound to celite was 50 times more susceptible to cleavage by kallikrein than HF in solution. High-M_r kininogen further enhanced the susceptibility to cleavage of the surface-bound HF by 10-fold (Fig. 1), and, therefore, surface plus high-M_r kininogen resulted in a 500-fold difference. However, high-M_r kininogen did not affect the cleavage of HF in solution by kallikrein (Fig. 1). Results identical to those seen here were obtained when similar concentrations of kaolin or suspensions of ellagic acid ranging from 10^{-6} to 10^{-3} M were substituted for celite. The observed enhancement of the cleavage of HF bound to celite in the presence of high M_r kininogen ranged from 250 to 500 in different experiments over a 1-year period, using different preparations of HF, kallikrein, and high-M_r kininogen.

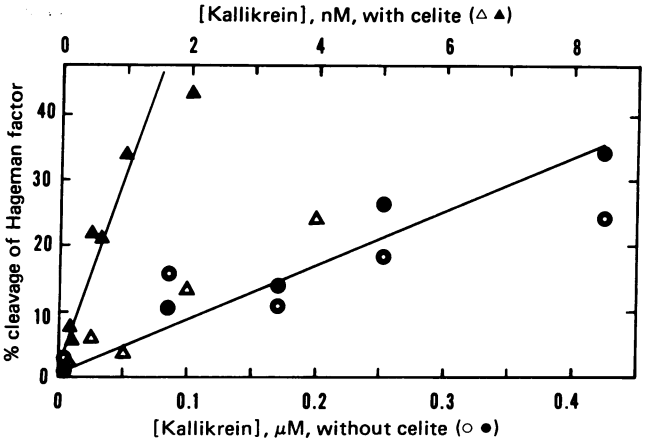

FIG. 1. Cleavage of celite-bound or soluble Hageman factor by plasma kallikrein in the presence (●,▲) or absence (○,△) of high-M_r kininogen. The final concentrations of reagents in the reaction mixture (0.1 ml) were 90 nM HF, 100 nM high-M_r kininogen (when present), celite (when present) at 5 mg/ml, and bovine serum albumin at 0.5 mg/ml. The 0.1 ml was composed of 0.06 ml of Tris-buffered saline, 0.02 ml of storage buffer, and 0.02 ml of 0.1 M TrisCl, pH 7.4. The reaction mixture was incubated at 37° for 9 min, and the reaction was terminated by adding to the tube 0.02 ml of a solution containing 5% $NaDodSO_4$ and 1% mercaptoethanol and then immediately placing the tube in a boiling water bath for 10 min.

Cleavage of HF by plasmin was studied in the presence of celite and high-M_r kininogen. Fig. 2 describes the percent of HF molecules cleaved during a 3-min incubation as a function of plasmin concentration. The upper and lower abscissas of Fig. 2 differ in plasmin concentration by two orders of magnitude. The data in Fig. 2 indicate that surface-bound HF is 100 times more susceptible to cleavage by plasmin than

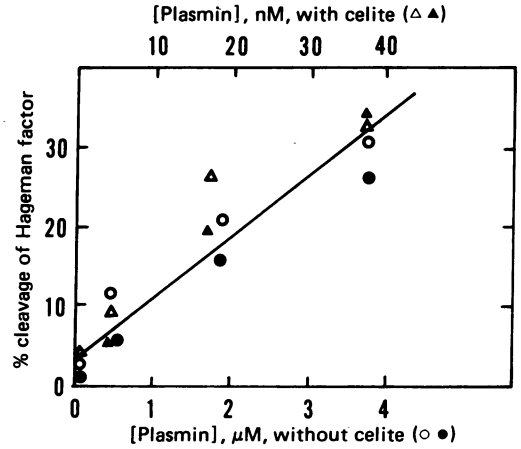

FIG. 2. Cleavage of celite-bound or soluble Hageman factor by plasmin in the presence (●,▲) or absence (○,△) of high-M_r kininogen. The final concentrations of reagents in the reaction mixture (0.15 ml) were 63 nM HF, 70 nM high-M_r kininogen (when present), celite (when present) at 2.2 mg/ml, and bovine serum albumin at 0.7 mg/ml. The 0.15 ml was composed of 0.1 ml of Tris-buffered saline, 0.02 ml of storage buffer, and 0.03 ml of 0.1 M TrisCl, pH 7.4. The reaction mixture was incubated at 37° for 3 min, and the reaction was terminated as described for Fig. 1.

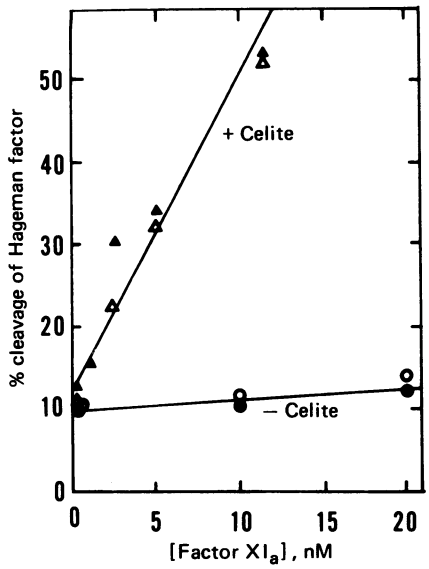

FIG. 3. Cleavage of celite-bound or soluble Hageman factor by coagulation Factor XI_a in the presence (●,▲) or absence (○,△) of high-M_r kininogen. The final concentrations of reagents in the reaction mixture (0.15 ml) were 63 nM HF, 70 nM high-M_r kininogen (when present), celite (when present) at 3.3 mg/ml, and bovine serum albumin at 0.7 mg/ml. The 0.15 ml was composed of 0.1 ml of Tris-buffered saline, 0.02 ml of storage buffer, and 0.03 ml of 0.1 M TrisCl, pH 7.4. The reaction mixture was incubated for 9 min at 37°, and the reaction was terminated as described for Fig. 1.

HF free in solution. High-M_r kininogen had no effect on the cleavage of surface-bound or soluble HF by plasmin. Similar results were obtained when celite was replaced by kaolin or suspensions of ellagic acid (1 mM) and when different preparations of each protein were used. Similar results were also obtained when no bovine serum albumin was present in the reaction mixtures.

Cleavage of Hageman Factor by Activated Factor XI. The influence of celite and high-M_r kininogen on the cleavage of HF by Factor XI_a is seen in Fig. 3. These data show that celite-bound HF was cleaved at least 30 times more readily than HF in solution by Factor XI_a. High-M_r kininogen did not affect these reactions (Fig. 3).

Cleavage of Hageman Factor by Trypsin. In studies similar to those described above (Figs. 1–3), the cleavage of HF by trypsin was determined. Celite-bound HF was 5 times more susceptible to tryptic cleavage than HF in solution. High-M_r kininogen did not affect the tryptic cleavage of either surface-bound or soluble HF in five different experiments employing different preparations of HF and trypsin.

DISCUSSION

The studies of the cleavage of ^{125}I-HF described here show that surface binding of human HF in the presence of high-M_r kininogen renders the molecule much more susceptible to limited proteolysis by human kallikrein, plasmin, or Factor XI_a by 500-fold, 100-fold, or 30-fold, respectively. High-M_r kininogen facilitated the action by kallikrein but not plasmin, Factor XI_a, or trypsin on surface-bound HF (32). High-M_r kininogen had no observable effect on the cleavage of HF in solution by kallikrein, plasmin, Factor XI_a, or trypsin. Recently it has been shown that when plasma containing ^{125}I-HF is added to a glass tube, thereby initiating contact activation, HF bound to the glass surface is rapidly cleaved to give polypeptide products such as those described here (20). These polypeptides represent

Table 1. Relative potency of kallikrein, plasmin, Factor XI_a, and trypsin in the cleavage of surface-bound and soluble Hageman factor

	Surface-bound HF		HF in solution	
Protease	Concentration,* nM	% of maximum plasma concentration†	Concentration,* nM	% of maximum plasma concentration†
Trypsin	0.10	—	0.42	—
Kallikrein‡	0.35	0.07	120	24
Plasmin	4.0	0.3	400	13
Factor XI_a	2.4	5.0	75	150

* The concentration of enzyme required to cleave 10% of the available HF in 9 min at 37° was calculated from *Results*.

† This percent was calculated on the basis of the respective plasma concentrations of prekallikrein, plasminogen, and Factor XI of 0.5 μM, 3.0 μM, and 0.05 μM.

‡ The value for kallikrein in the cleavage of surface-bound HF is that observed in the presence of high-M_r kininogen. In the absence of high-M_r kininogen, kallikrein was $^1/_{10}$ as potent. For the other enzymes, the presence of high-M_r kininogen did not affect the rates of cleavage of HF.

the two major forms of activated HF, a single chain 28,000-M_r molecule and a disulfide-linked two-chain 80,000-M_r molecule containing a heavy chain of 52,000 M_r in addition to the 28,000-M_r polypeptide (20). These observations on the rapid cleavage of ^{125}I-HF in plasma and the results reported here support the hypothesis that surface binding of HF causes a conformational change in the molecule which results in a structure that is much more susceptible to proteolytic activation (32). Fig. 4 shows a simple scheme depicting this mechanism. This hypothesis is particularly attractive in helping to explain the role of surface in the activation of HF because studies using [^{3}H]diisopropyl phosphorofluoridate as a quantitative active site titrant of surface-bound HF suggested that no detectable (<0.4%) nonproteolytic activation of HF results simply from surface binding (16–19).

A proper assessment of possible reciprocal activation mechanisms involving HF and prekallikrein (plasminogen proactivator), Factor XI, and plasminogen requires quantitative descriptions and analyses of each of the individual reactions involved. In this regard it is important to note the relative potency of each enzyme required to cleave 10% of available HF molecules as calculated from the data in Figs. 1–3. In the cleavage of surface-bound HF, kallikrein is almost as potent as trypsin, while plasmin and Factor XI_a are each an order of magnitude less potent than kallikrein (Table 1). In the cleavage of HF in solution, trypsin was 160 to 600 times more potent than the various plasma enzymes (Table 1). In order to evaluate the potential relevance of the observed cleavage of HF to events that could occur in plasma, Table 1 also lists the percentage of the normal plasma concentration that this would represent for each plasma enzyme. For example, activation of 0.04% of the total prekallikrein in plasma (0.5 μM) would give 0.2 nM kallikrein, which is reasonably potent in the cleavage of surface-bound HF. Activation of 0.1% of the total plasminogen in plasma could also measurably effect this reaction. Hence, concentrations that might be regarded as traces of kallikrein or plasmin in plasma could be quite significant in surface-dependent proteolytic activation of HF.

At least one secondary pathway that is independent of prekallikrein must exist for the activation of HF because contact activation reactions as well as the cleavage of surface-bound

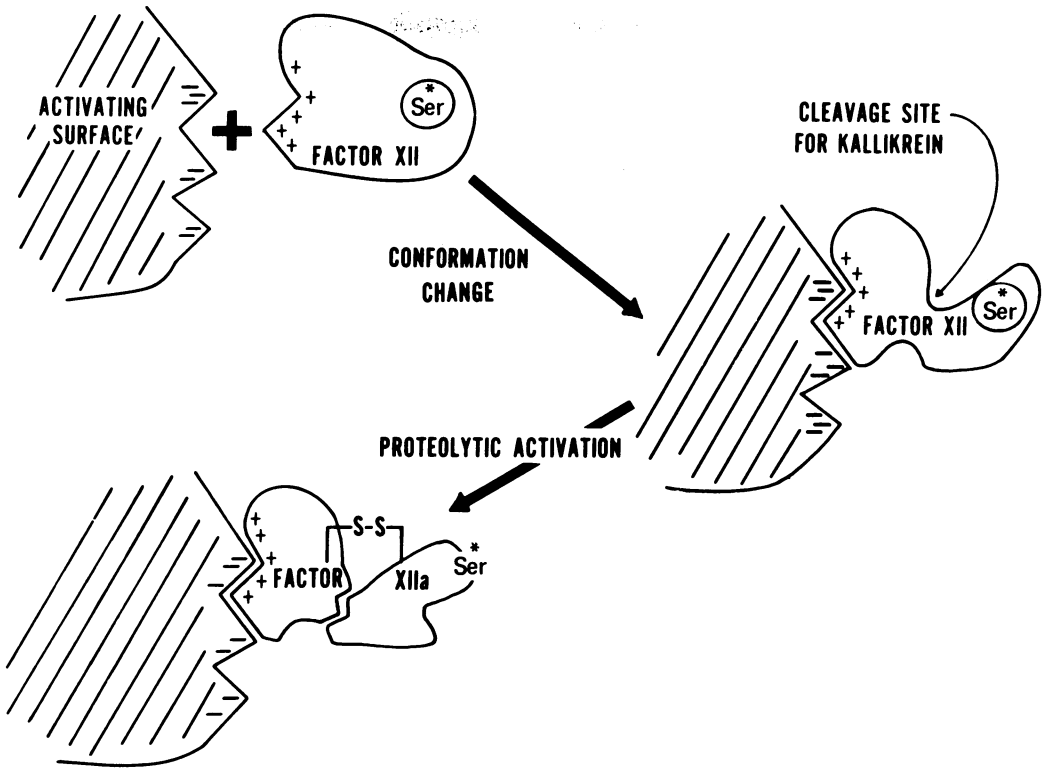

FIG. 4. Schematic model depicting the contribution of surface to the activation of Hageman factor (Factor XII). In this drawing, a molecule of Factor XII (HF), which is a serine protease zymogen, combines with a negatively charged surface, inducing a conformational change in the molecule. This conformational change does not *per se* activate the molecule, but rather it renders Factor XII much more susceptible to proteolytic activation by kallikrein (or by plasmin, Factor XI_a, and other proteases). This results in a surface-bound Factor XII_a (activated HF) molecule of 80,000 M_r containing two polypeptide chains linked by a disulfide bond. Not shown here is the possibility that cleavage of Factor XII sometimes occurs outside the disulfide bond (20) and results in the formation of a 28,000-M_r form of Factor XII_a that is free to diffuse away from the surface. Each form of Factor XII_a is a potent activator of prekallikrein, while only the surface-bound 80,000-M_r form of Factor XII_a in the presence of high-M_r kininogen is a potent activator of Factor XI (35).

^{125}I-HF are severely retarded but not absent in prekallikrein-deficient plasma (7, 8, 20). Given the high sensitivity of surface-bound HF to cleavage of plasmin (Table 1), it is tempting to speculate that a secondary pathway could involve plasmin.

In contrast to the potency of kallikrein and plasmin in the cleavage of surface-bound HF, rather large percentages of the available Factor XI in plasma would have to be activated in order to have comparable influence (Table 1). Furthermore, for the cleavage of HF in solution in plasma, the fact that 13–24% of the available plasma plasminogen or prekallikrein needs to be activated to accomplish extensive cleavage (Table 1) suggests that the proteolytic activation of HF in solution by either of these proteases may not be a particularly effective pathway in normal plasma.

Several observations are consistent with the hypothesis that the major pathway for activation of HF during contact activation involves limited proteolysis of surface-bound HF by kallikrein. First, the data reported here emphasize the high sensitivity of surface-bound HF to proteolytic activation (Table 1). Second, studies of the cleavage of surface-bound and soluble ^{125}I-HF in plasma during contact activation demonstrated that surface-bound HF is rapidly cleaved under conditions in which soluble HF is not cleaved (20). Third, the surface-bound single chain form of HF does not react with the active site titrant diisopropyl phosphorofluoridate (16–19) and does not exhibit esterase activity (19). Fourth, studies of the binding and cleavage of ^{125}I-prekallikrein in plasma during contact activation showed that kallikrein is rapidly generated and that most of the kallikrein is in solution (31; S. D. Revak, B. N. Bouma, C. G. Cochrane, and J. M. Griffin, unpublished data). Hence, soluble kallikrein, which is potent in the cleavage of surface-bound HF, is rapidly generated and readily available in plasma during contact activation. Fifth, the correction of the coagulation defect of prekallikrein-deficient plasma requires the addition of enzymatically active kallikrein as well as kaolin (8, 34, 35). Thus, reciprocal proteolytic activation of HF and prekallikrein (20, 21, 32, 35) probably represents the major mechanism responsible for contact activation of HF.

The critical question remains; what can provide the triggering proteolytic activity that initiates the cycle of reciprocal proteolytic activation of surface-bound HF and prekallikrein? Only a small trace of active HF or kallikrein or perhaps another plasma or cellular protease would be needed to initiate the system. Several distinct possibilities can be considered. Plasma might always contain very low levels of active proteases which engage in an omnipresent low-grade expression of plasma proteolytic pathways. This hypothesis, which remains unproven, has been discussed with respect to fibrinogen turnover (36) and to the alternate pathway of complement (37). The active proteases might be secreted by tissue into the intravascular space under normal circumstances or as a result of injury or foreign stimuli. Alternatively, HF or prekallikrein might be "active zymogens," that is, either one might possess an inherent enzymatic activity analogous to trypsinogen (38, 39) procarboxypeptidase (40), or Factor VII (41). The observation that HF does take up [^{3}H]diisopropyl phosphorofluoridate over a period of hours is consistent with this possibility (16, 17).

The support and advice of Drs. Charles G. Cochrane and Bonno N. Bouma are gratefully acknowledged. The skillful technical work of

Kevin Bickford, Gregory Beretta, and Alice Kleiss is most appreciated. This is publication no. 1432 from the Department of Immunopathology, Scripps Clinic and Research Foundation. This work was supported in part by National Institutes of Health Grants HL-21544, HL-16411, and AI-07007; by the Council for Tobacco Research; and by a National Institutes of Health Research and Career Development Award to the author.

1. Nossel, H. L. (1964) *The Contact Phase of Blood Coagulation* (Blackwell Scientific Publishers, Oxford, England).
2. Ratnoff, O. D. (1966) *Prog. Hematol.* **5**, 204–245.
3. Margolis, J. (1958) *J. Physiol. (London)* **144**, 1–22.
4. Niewiarowski, S. & Prou-Wartelle, O. (1959) *Thromb. Diath. Haemorrh.* **3**, 593–603.
5. Iatrides, S. G. & Ferguson, J. H. (1961) *Thromb. Diath. Haemorrh.* **6**, 411.
6. Ratnoff, O. D. & Colopy, J. E. (1955) *J. Clin. Invest.* **34**, 602–613.
7. Hathaway, W. E., Belhasen, L. P. & Hathaway, H. S. (1965) *Blood* **26**, 521–532.
8. Wuepper, K. D. (1973) *J. Exp. Med.* **138**, 1345–1355.
9. Rosenthal, R. L., Dreslin, O. H. & Rosenthal, N. (1953) *Proc. Soc. Exp. Biol. Med.* **83**, 171–174.
10. Wuepper, K. D., Miller, D. R. & Lacombe, M. J. (1975) *J. Clin. Invest.* **56**, 1663–1672.
11. Saito, H., Ratnoff, O. D., Waldmann, R. & Abraham, J. P. (1975), *J. Clin. Invest.* **55**, 1082–1089.
12. Donaldson, V. H., Glueck, H. I., Miller, M. A., Movat, H. Z. & Habel, F. (1976) *J. Lab. Clin. Med.* **87**, 327–337.
13. Colman, R. W., Bagdasarian, A., Talamo, R. C., Scott, C. F., Seavey, M., Guimaraes, J. A., Pierce, J. V., Kaplan, A. P. & Weinstein, L. (1975) *J. Clin. Invest.* **56**, 1650–1662.
14. Schiffman, S. & Lee, P. (1975) *J. Clin. Invest.* **56**, 1082–1092.
15. Margolis, J. (1963) *Ann. N.Y. Acad. Sci.* **104**, 133–145.
16. Griffin, J. H. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 329.
17. Griffin, J. H. (1977) *Thromb. Haemostasis* **38**, 50.
18. Claeys, H., Van Hoef, B. & Collen, D. (1977) *Throm. Haemostasis* **38**, 12.
19. Fujikawa, K., Kurachi, K. & Davie, E. W. (1977) *Biochemistry*, **16**, 4182–4188.
20. Revak, S. D., Cochrane, C. G. & Griffin, J. H. (1977) *J. Clin. Invest.* **59**, 1167–1175.
21. Cochrane, C. G., Revak, S. D. & Wuepper, K. D. (1973) *J. Exp. Med.* **138**, 1564–1583.
22. Kaplan, A. P. & Austen, K. F. (1971) *J. Exp. Med.* **133**, 696–712.
23. Burrowes, C. E., Movat, H. Z. & Soltay, M. J. (1971) *Proc. Soc. Exp. Biol. Med.* **138**, 959–966.
24. Bagdasarian, A., Lahiri, B. & Colman, R. W. (1973) *J. Biol. Chem.* **248**, 7742–7749.
25. Griffin, J. H. & Cochrane, C. G. (1976) in *Methods in Enzymology*, ed. Lorand, L. (Academic, New York), Vol. **45**, pp. 56–65.
26. Weber, K., Pringle, J. R. & Osborn, M. (1972) in *Methods in Enzymology*, eds. Hirs, C. H. W. & Timasheff, S. N. (Academic, New York), Vol. 26, pp. 3–27.
27. McConahey, P. J. & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol.* **29**, 185–189.
28. Bouma, B. N. & Griffin, J. H. (1978) *J. Lab. Clin. Med.*, **91**, 148–155.
29. Bouma, B. N. & Griffin, J. H. (1977) *Thromb. Haemostasis* **38**, 136.
30. Bouma, B. N. & Griffin, J. H. (1977) *J. Biol. Chem.* **252**, 6432–6437.
31. Wiggins, R. C., Bouma, B. N., Cochrane, C. G. & Griffin, J. H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4636–4640.
32. Griffin, J. H. & Cochrane, C. G. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2554–2558.
33. Revak, S. D., Bouma, B. N., Cochrane, C. G. & Griffin, J. H. (1978) *J. Exp. Med.*, **147**, 719–729.
34. Saito, H., Ratnoff, O. D. & Donaldson, V. H. (1974) *Circ. Res.* **34**, 641–651.
35. Meier, H. L., Pierce, J. V., Colman, R. W. & Kaplan, A. P. (1977) *J. Clin. Invest.* **60**, 16–31.
36. Nossel, H. L., Yudelman, I., Canfield, R. E., Butler, V. P., Jr., Spanondis, K., Wilner, G. D. & Qureshi, G. D. (1974) *J. Clin. Invest.* **54**, 43–53.
37. Fearon, D. T. & Austen, K. F. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1683–1687.
38. Kay, J. & Kassell, B. (1971) *J. Biol. Chem.* **246**, 6661–6668.
39. Robinson, N. C., Neurath, H. & Walsh, K. A. (1973) *Biochemistry* **12**, 420–426.
40. Behnke, W. D. & Vallee, B. L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2442–2446.
41. Radcliffe, R. & Nemerson, Y. (1976) *J. Biol. Chem.* **251**, 4797–4802.