Proc. Natl. Acad. Sci. USA
Vol. 73, No. 8, pp. 2554–2558, August 1976
Biochemistry

Mechanisms for the involvement of high molecular weight kininogen in surface-dependent reactions of Hageman factor

(blood coagulation/factor XII/prekallikrein/factor XI/fibrinolysis)

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Communicated by Frank J. Dixon, April 8, 1976

ABSTRACT **The mechanisms by which human high molecular weight kininogen (HM_rK) contributes to the surface-dependent activation of the Hageman factor systems have been studied. The ability of various mixtures of purified human Hageman factor (coagulation factor XII), HM_rK, prekallikrein, and kaolin to activate coagulation factor XI was determined with factor XI_a (activated factor XI) clotting assays. Hageman factor, HM_rK and prekallikrein were required for maximal rates of activation of factor XI. A certain optimal mixture of purified Hageman factor, HM_rK, prekallikrein, and kaolin gave the same rapid initial rate of activation of purified factor XI as an equivalent aliquot of factor XI-deficient plasma. This suggests that potent, surface-mediated activation of factor XI in plasma is explicable in terms of Hageman factor, HM_rK, and prekallikrein.**

By studying separately some of the surface-dependent reactions involving Hageman factor, it was found that HM_rK accelerated by at least an order of magnitude the following reactions: (*i*) the activation of factor XI by activated Hageman factor; (*ii*) the activation of prekallikrein by activated Hageman factor; and (*iii*) the activation of Hageman factor by kallikrein. Stoichiometric rather than catalytic amounts of HM_rK gave optimal activation of factor XI. These results are consistent with the hypothesis that HM_rK and Hageman factor form a complex on kaolin which renders Hageman factor more susceptible to proteolytic activation by kallikrein and which facilitates the action of activated Hageman factor on its substrate proteins, factor XI and prekallikrein.

Exposure of human plasma to certain surfaces such as glass or kaolin initiates a group of reactions known as the contact activation phase. These reactions include the activation of the intrinsic blood coagulation sequence (1), the kinin-forming pathway (2), the plasma fibrinolytic system (3), and the generation of permeability factors found in diluted plasma (4). Identification of proteins involved in the contact activation reactions has been based on the discovery of human plasmas which are functionally defective in contact activation and which lack a specific plasma protein. In 1955, Ratnoff and Colopy described a plasma which did not clot in glass tubes and, thus, Hageman factor (HF; coagulation factor XII) was discovered (5). For some time it was thought that HF is activated nonproteolytically upon binding to an activating surface, and then activated Hageman factor (HF_a) activates factor XI (6, 7). Later, it was shown that kallikrein could activate HF by proteolysis (8). This led to the identification of Fletcher plasma (9, 10) as being deficient in prekallikrein (11, 12). And it also led to the formulation of a more complex mechanism involving reciprocal activation of prekallikrein to kallikrein and HF to HF_a because it was shown with purified proteins that kallikrein specifically cleaves HF to give a 28,000 M_r fragment which itself is a potent proteolytic activator of prekallikrein (8, 11).

Abbreviations: HF, Hageman factor; HF_a, activated Hageman factor; HM_rK, high molecular weight kininogen; factor XI_a, activated factor XI; DiP-F, diisopropylfluorophosphate; PK, prekallikrein.

Most recently, plasma deficient in a high-molecular-weight form of kininogen (HM_rK) (13), alternatively known as Fitzgerald trait (14, 15), Williams trait (16), or Flaujeac trait (13), has been shown to be severely defective in the contact activation reactions. Other work with partially purified factor XI and HF led to the suggestion that an unidentified factor is involved in the activation of factor XI by HF (17), and this factor was later suggested to be HM_rK (18). However, no specific role for HM_rK in the contact activation reactions has been demonstrated. The studies presented here identify specific functional roles played by HM_rK in contact activation reactions and they also suggest that the contact phase of the coagulation in plasma can be explained in terms of molecular interactions involving HF, prekallikrein, HM_rK, factor XI, and an activating surface (19).

MATERIALS AND METHODS

Human HF and HM_rK were isolated from fresh plasma containing citric acid-dextrose as described elsewhere (ref. 20, and manuscript in preparation) and were judged greater than 95% homogeneous on polyacrylamide gels run in the presence of sodium dodecyl sulfate (21). Prekallikrein and factor XI were partially purified and generously provided by Dr. Richard Ulevitch. For experiments described in Table 2, factor XI was purified to greater than 95% homogeneity as judged on polyacrylamide gels (Bouma and Griffin, manuscript in preparation).

The HF coagulation assay based on two-stage kaolin-activated partial thromboplastin times using HF-deficient plasmas has been described elsewhere (20). Similar two-stage assays for factor XI, prekallikrein, and HM_rK were used. Fitzgerald trait plasma and factor XI-deficient plasma (<1% factor XI) were helpfully obtained from Mr. George King, George King Biomedical, Salem, N.H. Washington trait plasma was generously supplied by Dr. V. Donaldson and Fletcher trait plasma was obtained from Dr. C. Abildgaard. Mrs. Gunda Hiatt donated HF-deficient plasma. One unit of clotting activity is defined as the activity present in 1 ml of a citrated normal plasma pool. Protein concentration was determined by the Lowry method (22) with bovine serum albumin (Sigma) as a reference, and HF and prekallikrein concentrations were also measured immunologically using radial immunodiffusion (23). The clotting activities of the reagents were 80 ± 15 units/mg of HF activity, and 10 ± 2 units/mg of HM_rK activity. Solutions of HF and HM_rK contained no detectable factor XI or prekallikrein activity (<0.1%). HF at 9 ± 1 units/ml contained 0.36 units/ml of HF_a activity in the absence of kaolin as measured in siliconized glass tubes, and less than 0.02 units/ml of HM_rK activity. HM_rK at 0.8 ± 0.1 units/ml contained no HF activity (<0.001 units/ml). Solutions of factor XI and prekallikrein contained no detectable HF or HM_rK activity (<0.01%). The

Table 1. Activation of factor XI by mixtures of HF, HM_rK, prekallikrein (PK), and kaolin*

Reagents				Factor XI_a generated (clotting units/ml)
Kaolin	HF	HM_rK	PK	
+	+	+	+	0.34
+	—	—	—	0†
—	+	+	+	0.001
+	—	+	+	0.001
+	+	—	+	0.026
+	+	+	—	0.030
+	+	—	—	0.006
+	—	—	+	0.005
+	—	+	—	0.007

* The + or − sign indicates the presence or absence of the indicated reagent in a mixture containing factor XI at 0.83 units/ml which was incubated 8 min at 37° and then assayed for factor XI_a activity. The concentrations of HF, HM_rK, and PK, when present in the mixture, were 18, 14, and 6 μg/ml, respectively. Kaolin concentration was 3.6 mg/ml. The solution contained 0.10 M NaCl, 0.05 M Tris·Cl at pH 7.4. The observed clotting times varied from >300s for factor XI alone to 118s for 0.34 clotting units/ml.

† The background activity for factor XI alone plus kaolin was 0.006 clotting units/ml and this value was subtracted from each value in order to define the net activation of factor XI.

stock solution of factor XI at 10 ± 1 units/ml and 0.86 mg of protein per ml had less than 0.04 units/ml of activated factor XI (XI_a) and 0.8 ± 0.1 units/ml of prekallikrein clotting activity measured in Fletcher plasma. The stock solution of prekallikrein at 1.4 units/ml and 1.31 mg of protein per ml had 0.06 units/ml of factor XI activity.

HF was labeled with ^{125}I using the chloramine-T method (24) and radioactivity was measured with a Packard 5320 gamma spectrometer. The ^{125}I-HF contained 2–4 μCi/μg and exhibited full clotting activity after the labeling procedure. ^{125}I-HF was diluted with unlabeled HF for studies of the cleavage of ^{125}I-HF by kallikrein.

Kallikrein was obtained by mixing 60 μl of the stock prekallikrein solution with 0.3 μg of trypsin (TRTPCK, Worthington Biochem., N.J.) in a final 75 μl volume of 0.01 M Tris·Cl at pH 7.4, 0.15 M NaCl. After 40 min at 37°, 200 μg of ovomucoid trypsin inhibitor was added because this inhibits trypsin but not kallikrein or active HF. Kallikrein activity was assayed spectrophotometrically by the hydrolysis of Bz-Arg-OEt (25) or, alternatively, of the tripeptide Bz-Pro-Phe-Arg-paranitroanilide (Pentapharm A. G., Basel) (26).

RESULTS

The requirement for HF, HM_rK, prekallikrein, and kaolin for activation of coagulation factor XI

The ability of various combinations of purified HF, HM_rK, prekallikrein, and kaolin to activate partially purified factor XI in an incubation mixture was studied. The results seen in Table 1 show that efficient conversion of factor XI to factor XI_a requires HF, HM_rK, prekallikrein, and kaolin. In other reconstitution experiments, it was found that bradykinin from 5 to 500 ng would not replace 0.5 μg of HM_rK, that appropriate amounts of kallikrein plus HM_rK would not substitute successfully for HF, and that cephalin would not substitute for kaolin.

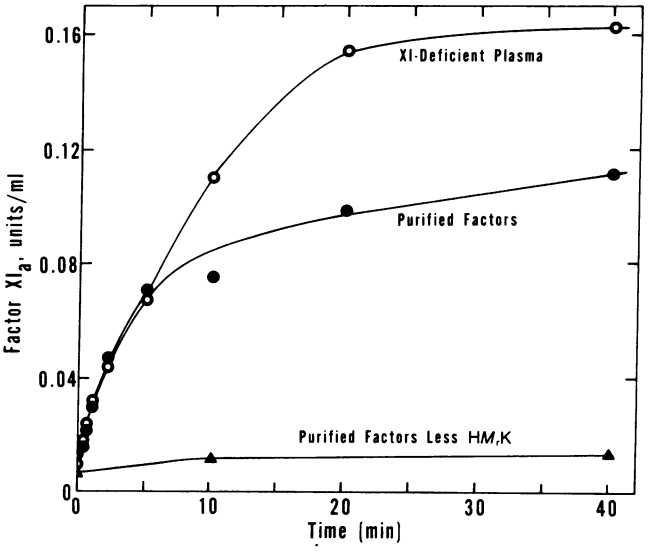

FIG. 1. Activation of factor XI by the combination of purified HF, prekallikrein (PK), and HM_rK or by the combination of purified HF and PK, or by an equivalent aliquot of factor XI-deficient plasma. To generate the factor XI activator with purified factors, the preincubation mixture contained, in order of addition, 25 μl of Tris-saline buffer (0.1 M Tris·Cl, 0.05 M NaCl at pH 7.5), 5 μl of HF (0.5 μg), 5 μl of PK (0.15 μg), either 5 μl of HM_rK (0.4 μg) or 5 μl of storage buffer (0.15 M NaCl, 0.5 mM EDTA, 6 mM acetate at pH 5.0), and 10 μl of kaolin (100 μg). After 5 min at 37°, 200 μl of Tris-saline buffer was added, and a 50 μl aliquot was withdrawn. To this 50 μl aliquot was added 5 μl of factor XI (0.91 clotting units/ml of final concentration), and after various times at 37° as indicated, this activation mixture was assayed for factor XI_a. To generate the factor XI activator using factor XI-deficient plasma, the preincubation mixture contained 20 μl of buffer (0.1 M Tris·Cl, 0.05 M NaCl at pH 7.5), 20 μl of factor XI-deficient plasma (containing 0.5 μg of HF, 0.2 μg of PK, and approximately 1.6 μg of HM_rK), and 10 μl of kaolin (100 μg). After 5 min preincubation at 37°, the kinetics of factor XI activation were measured as described above.

The kinetics of factor XI activation were defined in experiments in which identical aliquots of factor XI were incubated for varying times, either with kaolin and a mixture of HF, HM_rK, and prekallikrein, or with kaolin and an aliquot of factor XI-deficient plasma which contained the same amount of HF and prekallikrein and excess HM_rK. The data seen in Fig. 1 show that the reconstituted mixture of purified factors on kaolin gave the same initial rate of factor XI activation as that given by an equivalent aliquot of factor XI-deficient plasma, which thus indicates that the ability of this mixture of purified HF, HM_rK, and prekallikrein to activate factor XI is equal to the ability of plasma to activate factor XI. The total amount of factor XI which became activated in the two different mixtures described in Fig. 1 was somewhat higher in the presence of plasma and was probably due to the higher amount of HM_rK. These data suggest that the initial rapid activation of factor XI which occurs during the first several minutes of the contact phase of coagulation in plasma can be accounted for in terms of the molecular interactions involving HF, HM_rK, prekallikrein, and factor XI with an activating surface. The data also suggest that HF is not sufficiently activated on a negatively charged surface in the absence or even in the presence of HM_rK to activate readily factor XI.

Stoichiometric requirement for HM_rK.

The amount of HM_rK in the reconstitution mixture of HF, prekallikrein, factor XI, and kaolin was varied and the amount of factor XI_a formed during a 5 min incubation was measured

Table 2. Activation of factor XI* by kaolin-bound, trypsin-activated HF_a in the absence and presence of HM_rK

Reagents in incubation mixture†	Factor XI_a generated (clotting units/ml)
Factor XI, HF	0‡
Factor XI, HF_a	0.003
Factor XI, HF_a, HM_rK	0.050
Factor XI, HF_a, HM_rK, PK	0.059

* The stock solution of factor XI used for these experiments contained factor XI which was judged >95% homogeneous on polyacrylamide gels and which contained 2.5 units/ml of factor XI activity and less than 0.04 units/ml of PK clotting activity.

† The indicated reagents were incubated for 5 min at 37° and an aliquot was withdrawn for determination of factor XI_a activity. The final concentration of each reagent in the incubation mixture, when present, was: 0.18 units/ml of factor XI, 11 μg/ml of HF or HF_a, 10 μg/ml of HM_rK, and 2.5 μg/ml of PK. Each mixture contained 0.8 mg/ml of kaolin. HF_a was obtained by treating 16 μg of HF on 1.2 mg of kaolin with 1.2 μg of trypsin in the presence of 40 μg of bovine serum albumin for 10 min at 37° (total volume was 300 μl); the reaction was stopped by addition of 60 μl (120 μg) of ovomucoid trypsin inhibitor.

‡ The background activity for factor XI plus HF was 0.008 units/ml and this value was subtracted from each value to define the net activation of factor XI.

to define whether HM_rK acted in catalytic or stoichiometric amounts. Our increasing the amount of HM_rK in the mixture from 0 to 0.6 μg gave a linear increase in the amount of factor XI_a formed and, above the optimal value of 0.6 μg of HM_rK, inhibition of factor XI activation occurred. Since the reaction mixture contained 0.5 μg of HF, this suggests that HM_rK exerted its effects on this HF-dependent reaction in a stoichiometric manner.

Similar studies of the variation of prekallikrein in the reconstitution mixture indicated that prekallikrein acted in catalytic amounts.

Mechanisms of action of HM_rK

After the demonstration that the contact activation reactions require HM_rK for rapid development (*vide supra*), we then examined possible mechanisms by which the HM_rK could enhance or participate in the activation of the HF-dependent reactions. Several possibilities were investigated: (*i*) HM_rK enhances the activation of factor XI by kaolin-bound, trypsin-activated HF_a; (*ii*) HM_rK affects the cleavage and activation of kaolin-bound HF by kallikrein; and (*iii*) HM_rK accelerates the activation of prekallikrein involving HF or by kaolin-bound, trypsin-activated HF_a.

Activation of factor XI

The importance of HM_rK for the activation of factor XI by trypsin-activated HF (HF_a) is shown by the data in Table 2. Neither HF nor HF_a alone activated factor XI in the absence of HM_rK whereas the presence of HM_rK increased the activation of factor XI by HF_a from 0.003 to 0.050 units/ml. The additional presence of prekallikrein gave minimal additional activation of factor XI, i.e., from 0.050 to 0.059 units/ml. Consequently, HM_rK under these conditions is exerting its action primarily on the activation of factor XI by HF_a.

Activation of factor XII

The specific cleavage of ^{125}I-HF by kallikrein was studied in order to define the possible role of HM_rK in this reaction.

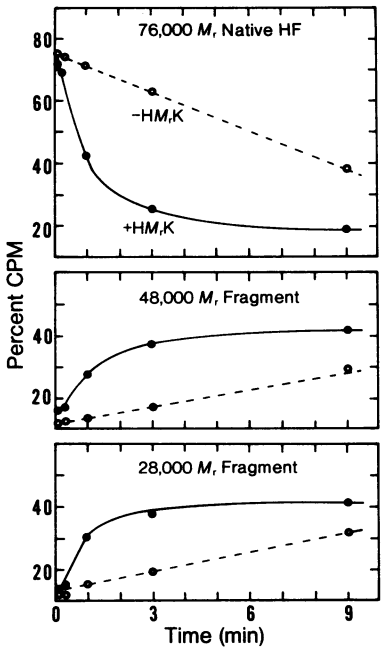

FIG. 2. Influence of (HM_rK) on the rate of cleavage of ^{125}I-HF by kallikrein. The total radioactivity corresponding to the peak at the indicated M_r was determined for various reaction times. The final concentrations of each reagent in the reaction mixture were: 14 μg/ml of HF, 14 μg/ml of HM_rK (when present), 0.5 μg/ml of kallikrein, and 1.7 mg/ml of kaolin. The solution contained 7 mM Tris-Cl, 0.15 M NaCl at pH 7.4. After a given incubation time at 37°, a 50 μl aliquot was withdrawn from the reaction mixture, and 100 μg of soybean trypsin inhibitor was added followed by 30 μl of 3% sodium dodecyl sulfate, and 3% of 2-mercaptoethanol.

^{125}I-HF and kallikrein were incubated with kaolin in the presence and absence of HM_rK, and, at varying incubation times, aliquots of the mixtures were analyzed for the extent of cleavage of ^{125}I-HF on 7½% sodium dodecyl sulfate gels. The native HF zymogen at 76,000 M_r was cleaved to give two polypeptides of 48,000 and 28,000 M_r. The integrated total radioactivity under each of the three peaks is shown as a function of incubation time in Fig. 2. Comparison in Fig. 2 of either the rates of disappearance of the native 76,000 M_r species or the rates of appearance of the 48,000 and 28,000 M_r fragments yields the observation that the presence of HM_rK increases the rate of cleavage of ^{125}I-HF by kallikrein by 8- to 11-fold.

Under similar conditions, the cleavage of ^{125}I-HF by kallikrein occurred at least one order of magnitude more slowly in the absence of kaolin than in its presence whether or not HM_rK was present.

Control experiments showed that the solution of kallikrein used for the experiments contained no prekallikrein. The specific esterase activity of kallikrein determined as hydrolysis of Bz-Arg-OEt was 52 μmol of ester hydrolyzed per min/mg of kallikrein. This specific esterase activity of kallikrein was not altered in the presence of HM_rK at 0.1–1.0 μg/ml.

Activation of prekallikrein

Because active HF is known to activate prekallikrein by limited specific proteolysis (8, 11), the possible involvement of HM_rK in the activation of prekallikrein by HF_a was studied. To obtain HF_a, HF on kaolin was exposed to mild tryptic hydrolysis with conditions that gave limited cleavage of HF. Ovomucoid trypsin inhibitor, which does not inhibit HF_a or kallikrein, was then used to neutralize the small amount of trypsin activity. The

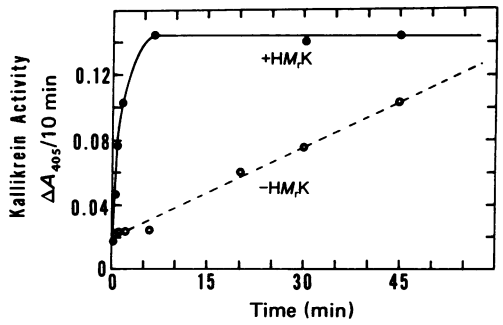

FIG. 3. Influence of HM_rK on the activation of PK by kaolin-bound, trypsin-activated HF. The final concentrations of reagents were: 3 μg/ml of HF_a, 7 μg/ml of PK, 0 or 10 μg/ml of HM_rK, and 0.6 mg/ml of kaolin. The solution contained 0.04 M Tris-Cl, 0.15 M NaCl at pH 7.4. After various indicated reaction times at 37°, a 50 μl aliquot was assayed at 20° for kallikrein activity.

data presented in Fig. 3 demonstrate that the rate of activation of prekallikrein by HF_a on kaolin was enhanced 20-fold in the presence of HM_rK. Significantly, the total final amount of prekallikrein which was activated was independent of HM_rK. Similar results were obtained in separate experiments when kallikrein activation was followed using assays of the hydrolysis of Bz-Arg-OEt. Control experiments showed that no prekallikrein activation occurred during similar 45 min incubations in the absence of HF_a whether or not HM_rK was present.

DISCUSSION

Mechanisms Involving HM_rK in HF-Contact Activation Reactions. Human plasma deficient in HF, prekallikrein, HM_rK, or factor XI lacks normal contact activation reactions (1, 12–16). Therefore, to define the molecular mechanisms responsible for contact activation, it was essential to characterize interactions between these proteins using highly purified factors in their precursor forms. We employed purified precursor HF and HM_rK, and partially purified prekallikrein and factor XI, and demonstrated that the combination of HF, prekallikrein, HM_rK, and factor XI with kaolin yields rapid activation of factor XI. Omission of any one of the reagents or kaolin resulted in about 1/10 the activation of factor XI. Notably, both HM_rK and prekallikrein are required for the efficient activation of factor XI by HF on kaolin. The observation that the initial rate of activation of factor XI by the purified recombined proteins equals the inital rate of activation of factor XI by an equivalent aliquot of plasma deficient in factor XI suggests that the contact phase of activation of the intrinsic coagulation pathway is explicable in terms of interactions between HF, prekallikrein, HM_rK, and factor XI in the presence of the activating surface.

Several specific roles of HM_rK in the contact activation reactions were defined by studying separately some of the individual reactions of the contact phase. First, the present results show that HM_rK is necessary for rapid activation of factor XI by trypsin-activated HF_a on kaolin. Second, the activation of prekallikrein by trypsin-activated HF_a on kaolin is accelerated by more than one order of magnitude in the presence of HM_rK. Third, the specific cleavage of ^{125}I-HF on kaolin by kallikrein is accelerated 10-fold in the presence of HM_rK. These reactions are depicted in Table 3. Thus, the influence of HM_rK in contact activation is not limited to only one particular reaction. Rather, HM_rK promotes the cleavage of two of the substrate proteins of HF_a, namely, factor XI and prekallikrein. Moreover, the rate of cleavage, i.e., activation (*vide infra*) of HF itself by kallikrein is enhanced by HM_rK. The existence of these multiple effects

Table 3. Specific sites of action of HM_rK in HF reactions

1. Factor XI $\xrightarrow[HF_a/kaolin]{HM_rK}$ Factor XI_a
2. PK $\xrightarrow[HF_a/kaolin]{HM_rK}$ Kallikrein
3. HF/kaolin $\xrightarrow[Kallikrein]{HM_rK}$ HF_a/kaolin

of HM_rK, together with the stoichiometric relation of HM_rK to HF observed for optimal factor XI_a generation, are consistent with the hypothesis (19) that HM_rK forms a stable complex with HF as well as with HF_a on kaolin and that this complex formation alters the conformation of HF such that it is much more susceptible to cleavage, i.e., to activation by kallikrein. Additionally, this complex formation would facilitate the action of HF_a on its substrate proteins.

Importance of Proteolytic Activation of Surface-Bound HF by Kallikrein. The relative importance of proteolytic versus nonproteolytic activation of HF which is surface-bound merits critical reassessment. The prevailing central concept of the mechanism of contact activation of HF has been that HF becomes HF_a without proteolysis upon binding to an activating surface. The observation that HF on kaolin which has been exposed to mild tryptic cleavage is a potent activator of factor XI suggests that the role of prekallikrein is to provide kallikrein which can rapidly activate HF by a single cleavage when HF is surface-bound in the presence of HM_rK.

Further support for the necessity of proteolysis of HF comes from preliminary experiments using diisopropylfluorophosphate (DiP-F). First, the ability of purified HF bound to kaolin to correct the partial thromboplastin time of HF-deficient plasma is not reduced by preincubation with DiP-F whereas the corrective ability of purified HF bound to kaolin in the presence of kallikrein and HM_rK is markedly diminished by preincubation with DiP-F. Second, purified HF bound to kaolin takes up very small amounts of [^{3}H]DiP-F whereas surface-bound HF which has been cleaved by trypsin or other proteases takes up 1 mol of [^{3}H]DiP-F per mol of HF in the 28,000 M_r fragment. These observations do not however rule out the possibility that a minor fraction of HF molecules become activated without proteolysis upon binding to an "activating" surface.

In this context, it is noteworthy that recent experiments by Revak *et al.* (27) have shown that normal amounts of ^{125}I-HF rapidly become surface-bound but are only very slowly cleaved when plasmas deficient in prekallikrein or HM_rK are exposed to glass. By contrast, ^{125}I-HF in normal plasma is rapidly bound to a glass surface and almost instantly cleaved. The slow rate of cleavage of ^{125}I-HF bound to a glass surface which occurs in plasma deficient in prekallikrein or HM_rK is in keeping with the observation that plasmas deficient in these proteins form contact activation products only after prolonged incubation with kaolin or glass if they are formed at all (9–16).

The fact that exposure to glass of plasma deficient in HM_rK resulted in ^{125}I-HF binding without cleavage (27) shows that HM_rK does not function by augmenting the amount of HF initially bound to an activating surface. Reconstitution of this plasma with purified HM_rK results in normal contact activation and in rapid cleavage of ^{125}I-HF as observed with normal human plasma (27). Thus, studies of the binding and cleavage of ^{125}I-HF in various deficient plasmas in glass tubes are consonant with an emphasis on proteolytic activation of surface-bound HF.

Current Hypothesis for Surface-Dependent Activation of HF. Our current working hypothesis for the mechanism of

contact activation of HF-dependent reactions is as follows. Exposure of plasma to an activating surface results in the formation of a surface-bound complex between HF and HM_rK which places HF into a conformation which is highly susceptible to proteolytic cleavage, i.e., activation, yielding surface-bound HF_a. This bound HM_rK/HF_a complex is a potent activator of factor XI and of prekallikrein. The newly formed kallikrein reciprocally activates more HF in other surface-bound HF/HM_rK complexes, thereby augmenting the amount of HF_a and, consequently, the activation of factor XI, prekallikrein, and plasminogen proactivator. Eventually, a 28,000 M_r portion of HF_a containing its active site diffuses off the surface into solution where it becomes inhibited by binding to C1 inh, the inhibitor of the activated first component of complement (28–30). Kallikrein also liberates kinins from HM_rK; and, interestingly, recent studies show that kallikrein acts on HM_rK much more rapidly than on low-molecular-weight kininogen (31, 32).

Certain critical aspects of contact activation remain unexplained by this simplified hypothesis—What molecules provide the initial triggering proteolytic activity for the HF_a-prekallikrein-kallikrein-HF cycle? Is it due to nonproteolytic activation of a small portion of the HF molecules which become bound or is it due to trace amounts of proteolytic activity omnipresent in plasma? Or might prekallikrein or HF actually possess a small inherent degree of activity similar to the low levels of enzymatic activity ascribed to procarboxypeptidase (33) or trypsinogen (34)?

The reconstitution of the contact activation reactions using purified proteins and the definition of several specific roles for HM_rK which are reported here (Table 3) help to further the elucidation of the molecular mechanisms responsible for the contact activation phase in plasma.

The skillful technical work of Gregory Beretta and Alice Kleiss is gratefully acknowledged, as are helpful discussions with Susan Revak and Drs. Bonno Bouma and Ted Zimmerman. This is manuscript no. 1171 from the Department of Immunopathology, Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, Calif. 92037. This work was supported by USPHS HL-16411, AI-07007 and John H. Griffin's Research and Career Development Award K04 HL00192.

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