SURFACE AND FLUID PHASE ACTIVITIES OF TWO FORMS OF ACTIVATED HAGEMAN FACTOR PRODUCED DURING CONTACT ACTIVATION OF PLASMA*

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During contact activation of plasma, cleavage of surface-bound Hageman factor (HF)[1] occurs at two closely situated sites, one within a disulfide loop, termed "site 1", and one outside the disulfide loop, termed "site 2" (1). The result of these cleavages is two forms of active HF (2) which we have given the names α-HF_a and β-HF_a. Fig. 1 defines these two forms based on their derivation (last column) and molecular characteristics (first four columns). Although it is known that contact activation of plasma will result in coagulation (via the intrinsic pathway), fibrinolysis, and kinin generation, a quantitative comparison of the ability of each form of HF_a to initiate these systems has not been made. We therefore undertook the studies reported here to investigate the relative ability of α-HF_a and β-HF_a to activate the HF substrates, prekallikrein and factor XI, in plasma during contact activation. The experiments were performed in whole plasma, rather than with purified components, to follow as closely as possible the events as they would occur during contact activation of plasma. It has been shown that the cleavage of prekallikrein is coincident with its activation by HF_a (3).[2] Activation of factor XI has also been shown to be associated with a proteolytic cleavage of its 80,000 mol wt subunit chains (3–5). Thus, by following the cleavage of radiolabeled prekallikrein and factor XI added to plasma, we could measure their rate of activation.

It has recently been reported from this laboratory that during contact activation, a large percentage of the factor XI and a small amount of the prekallikrein in plasma become surface bound (6). We have expanded upon these observations by investigating the influence of the surface on the cleavage of factor XI and prekallikrein and have followed the distribution of these

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[1] *Abbreviations used in this paper:* HF, Hageman factor; HF_a, activated Hageman factor; HMWK, high molecular weight kininogen; SDS, sodium dodecyl sulfate.

[2] B. N. Bouma. 1978. Manuscript in preparation.

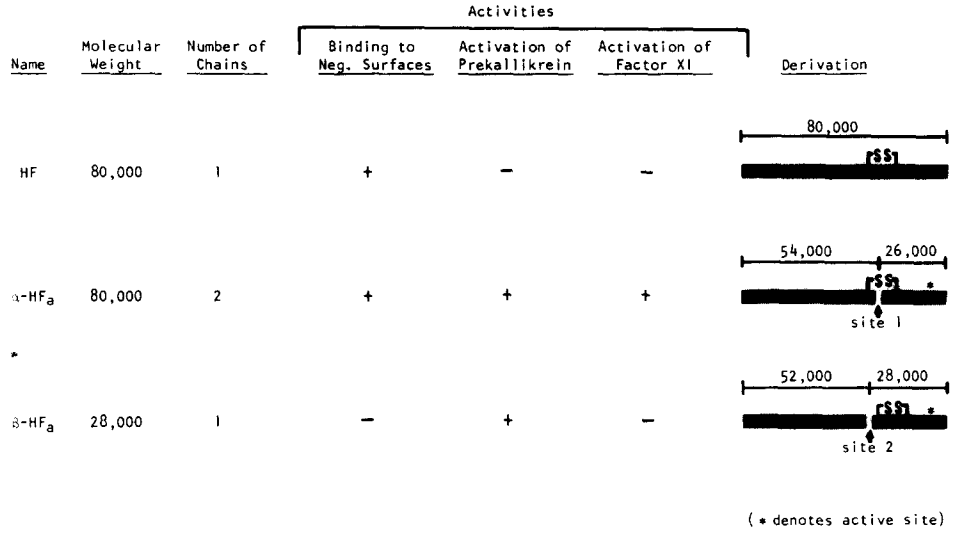

FIG. 1. Characteristics of HF and its activated forms produced during contact activation of plasma. Two forms of HF_a are defined by cleavage of the native zymogen at specific sites. The biologic activities of each and their molecular characteristics are as indicated.

proteins between the surface and fluid phase compartments before and after their interaction with HF_a.

Materials and Methods

Plasmas. Normal human plasma was pooled from 19 normal healthy adults using 1/6 vol of acid citrate dextrose as an anti-coagulant. Plasma deficient in high molecular weight kininogen (HMWK, plasma of Mr. Fitzgerald) was obtained from George King Biomedical (Salem, N. H.). Plasma deficient in prekallikrein was supplied by Dr. Charles Abildgaard. HF-deficient plasma was generously donated by Mrs. Gunda Hiatt.

Preparation and Radiolabeling of Proteins. HF and factor XI were isolated from human plasma as described elsewhere (7, 5). Isolation of prekallikrein was by methods similar to those utilized for factor XI purification (5), employing sequential chromatography on DEAE-, QAE-, and SP-Sephadex, concanavalin A-Sepharose, and a final separatory step of sucrose density ultracentrifugation.[3] Each was judged to be greater than 95% homogeneous when analyzed by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (SDS). HF having 80 ± 15 clotting U/mg protein was radiolabeled without loss of functional activity with ^{125}I and ^{131}I using the solid state lactoperoxidase method (8). The ^{125}I-HF contained 5.1–9.6 μCi/μg (1.2 to 1.9 μCi/μl) and the ^{131}I-HF 9.5 μCi/μg (0.57 μCi/μl). Factor XI was radiolabeled by the same method (8) with an uptake of 9 μCi/μl. After radiolabeling, the factor XI contained 3.8 clotting U of activity per ml. Prekallikrein, having a specific clotting activity of 19 U/mg, was radiolabeled with ^{125}I by the chloramine T method of McConahey and Dixon (9). It contained 9.1 μCi/μg (0.29 μCi/μl).

Contact Activation. Whole plasma, rather than isolated individual components, was chosen as the medium for the experiments to approximate conditions under which contact activation presumably occurs in vivo. In this way, not only the various components of the contact system would be present, but the inhibitors as well. The contact activation system used in these studies was chosen for its ability to provide a simple method of activating the enzymes of interest under conditions where the kinetics of activation could be followed and special procedures, such as removal of supernates and washing of surfaces, could be easily and rapidly accomplished. The

[3] B. N. Bouma and J. H. Griffin. 1978. Manuscript in preparation.

method consisted of the addition of a radiolabeled protein to plasma, an appropriate dilution, and the incubation of a sample in a glass tube. The radiolabeled protein added did not increase the plasma content of that protein by more than 20%. The plasma was diluted 1/6 in all experiments except, as noted, where complete activation of HF was required, in which case the dilution was 1/25. The diluent employed was Tris-buffered saline (0.01 M Tris, 0.15 M NaCl, pH 7.6). 20-μl aliquots were placed in 12 × 75 mm new, unwashed borosilicate glass tubes and shaken at room temperature for varying times. The procedure thereafter varied with the experiments and will be described in more detail below. In general, however, after 5 min of incubation, 50% of the HF was activated, with approximately equal amounts of α-HF_a and β-HF_a being formed. This corresponds to 0.04 μg HF_a per tube. Separation of the α- and β-forms of HF_a was accomplished by removal of the supernate from the tube. ("Supernate" is used here to refer to the plasma in the glass tube after incubation.) Previous reports have shown that under these conditions of activation, α-HF_a will be surface bound and β-HF_a will be found in the supernate (1, 2).

SDS-Polyacrylamide Gel Electrophoresis. Samples were prepared for electrophoresis by the addition of 50 μl of 4% SDS in 0.01 M sodium phosphate buffer pH 7.0 containing (except where noted) 2% β-mercaptoethanol. After an immediate 3 min incubation in a boiling water bath, sucrose was added to a 10% final concentration and a trace of bromophenol blue was included to serve as a tracking dye. Electrophoresis was carried out on 4.5 × 80 mm 7% polyacrylamide gels containing 0.1% SDS according to the method of Weber and Osborn (10) until the tracking dye was within 1 cm of the bottom of the tube. The gels were then removed from the tubes, sliced into 1.2-mm sections and counted for radioactivity in a model 1195 Searle Analytic Gamma counter (Searle Analytic Inc., San Diego, Calif.). Greater than 90% of the radioactivity applied to the gels was recovered.

Immunologic Quantitation of Kallikrein. Kallikrein was quantitated by the radial immunodiffusion technique of Mancini et al. (11) using a 1/10 dilution of goat antiserum against prekallikrein. The monospecific antibody was produced by the weekly injection of 60–80 μg purified human prekallikrein in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich) into multiple subcutaneous sites on the back of a 1-yr old goat. Serum collected after the 4th wk gave a single line of precipitation against normal human plasma when tested in double immunodiffusion tests and no line when tested against plasma deficient in prekallikrein.

Buffer. The buffer used for washing the surface-bound proteins was phosphate-buffered saline (0.01 M sodium phosphate, 0.15 M NaCl, pH 7.0).

Results

Cleavage of Prekallikrein and Factor XI in Plasma During Contact Activation. The kinetics of cleavage of prekallikrein and factor XI during contact activation of plasma was determined for the system to be used throughout these studies. ^{125}I-prekallikrein and ^{125}I-factor XI were added to separate samples of normal human plasma. A 1/6 dilution was made of the plasmas and 20-μl samples of each were incubated in glass tubes for varying time periods. To stop the reactions and elute any surface-bound material, 50 μl of an SDS-mercaptoethanol solution was added. The extent of cleavage of the radiolabeled proteins was assessed by electrophoresis on SDS-polyacrylamide gels. Prekallikrein and factor XI, run under reducing conditions, both migrate as polypeptides of approximately 80,000 mol wt. Upon the exposure of plasma to glass, ^{125}I-prekallikrein is cleaved to yield primarily a 47,000 mol wt fragment; presumably the remainder of the molecule is unlabeled by the procedure used. ^{125}I-factor XI in plasma exposed to glass is cleaved into fragments of 47,000 and 33,000 mol wt. Typical cleavage patterns for these two proteins are shown in Fig. 2. HF of 80,000 mol wt and its 52,000 and 28,000 mol wt cleavage fragments were included in the gels as molecular weight standards for comparison. The percent of radiolabel in each gel which appeared at a mol wt of 80,000 was calculated. With the percent present in the 80,000 peak at a zero time point

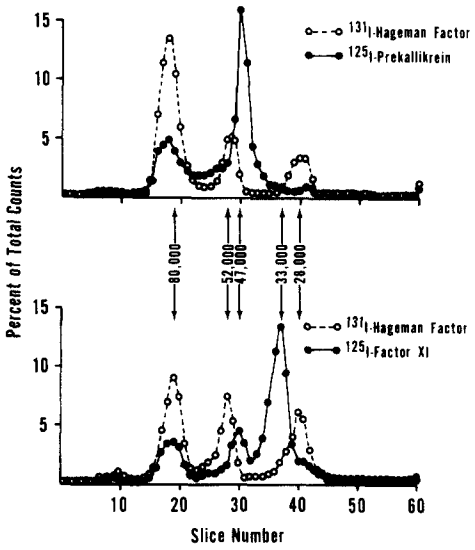

FIG. 2. Analysis by SDS-polyacrylamide gels of cleavage of ^{125}I-prekallikrein and ^{125}I-factor XI in plasma. Samples of diluted normal plasma containing ^{131}I-HF and either ^{125}I-prekallikrein or ^{125}I-factor XI were incubated in glass tubes. SDS-mercaptoethanol was added to the sample containing ^{125}I-prekallikrein after 2 min incubation and to the sample containing the ^{125}I-factor XI after 5 min incubation. The samples were then subjected to electrophoresis in 7% polyacrylamide gels containing 0.1% SDS. The gels were sliced and counted for radioactivity. Molecular weights are based on the known weights of the HF fragments.

taken as 100%, the percent remaining at 80,000 (i.e., percent uncleaved) was computed and, by subtracting this value from 100%, the data were expressed as percent of molecules cleaved. The kinetics of cleavage for ^{125}I-prekallikrein and ^{125}I-factor XI are shown in Fig. 3.

Cleavage of Surface-Bound Factor XI and Prekallikrein by Surface-Bound α-HF$_a$. To study cleavage of factor XI on a surface by surface-bound α-HF$_a$, 20-μl aliquots of a $^1/_6$ dilution of normal plasma containing ^{125}I-factor XI were shaken in glass tubes. At the end of a 2 min incubation period, the supernate was removed and the tube washed three times with 200 μl buffer. Preliminary experiments showed that in 2 min sufficient HF became bound and cleaved (activated) to yield measurable rates of factor XI cleavage. This incubation period was also sufficient to bind 62% of the ^{125}I-factor XI; 44% of that which was bound, was cleaved. 50 μl of an SDS-mercaptoethanol solution was added to one tube, thereafter called the 0 time sample. The remaining tubes received 2 ml of buffer and were incubated with shaking for varying times. In the presence of 2 ml of buffer, any α-HF$_a$ which might elute off the surface, or β-HF$_a$ which was released, would become rapidly diluted (a minimum of 600-fold) and the probability of any interaction between them and surface-bound factor XI would be greatly diminished. After the desired incubation times, the 2 ml of buffer was removed and 50 μl of the SDS-mercaptoethanol solution was added. All samples were analyzed by SDS-polyacrylamide gel electrophoresis for the amount of cleavage of ^{125}I-factor XI. The 0 time point was taken as 0% cleavage

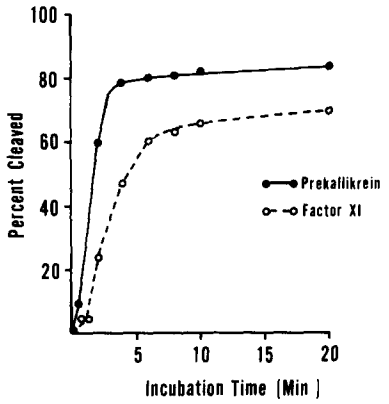

FIG. 3. Cleavage of prekallikrein and factor XI in plasma during contact activation. Samples of diluted normal plasma containing either ^{125}I-prekallikrein or ^{125}I-factor XI were incubated in glass tubes for varying times. SDS-mercaptoethanol was added to stop the reactions and the samples were analyzed by electrophoresis on SDS-polyacrylamide gels for cleavage of the ^{125}I-prekallikrein and ^{125}I-factor XI. The percent of prekallikrein and factor XI cleaved was determined in both cases by the disappearance of ^{125}I from the 80,000 mol wt peak of native material.

(i.e., the 44% cleavage observed after the initial 2 min incubation which could not be assumed to be due strictly to surface reactions was subtracted). The results, shown as the dashed line in Fig. 4 indicate that significant cleavage occurs over a 20 min time period. Since fluid phase contributions were minimized by dilution during the 20 min of incubation, the observed cleavage was essentially caused by the action of surface-bound α-HF_a on surface-bound factor XI.

Since prekallikrein binding to the surface is quite weak (6), a different experimental system was employed to investigate its cleavage on the surface. ^{125}I-prekallikrein was added to HF-deficient plasma. After a 1/6 dilution, 20-μl aliquots were incubated in glass tubes for 5 min, the supernates removed, and the tubes washed three times with 200 μl buffer. This procedure resulted in the binding of 10% of the ^{125}I-prekallikrein and since HF-deficient plasma was used, no cleavage occurred during these 5 min. After the washing of the bound ^{125}I-prekallikrein, 20 μl of a 1/6 dilution of prekallikrein-deficient plasma was added as a source of HF to each tube for 20 s, diluted out by the addition of 2 ml buffer which was then immediately removed and replaced with a fresh 2 ml of buffer. This step was designed to allow sufficient HF to become bound to the surface and activated and then to remove all nonbound proteins and allow the incubation to proceed under conditions where any proteins being released from the surface would be prevented by dilution from entering into the cleavage reaction occurring on the surface. Timing was started from the moment of addition of the second 2 ml of buffer. At the end of the desired incubation time, the buffer was removed and the surface-bound proteins eluted by the addition of SDS-mercaptoethanol. After analysis on SDS-polyacrylamide gels, the percent of ^{125}I-prekallikrein cleaved was determined. The results are shown as the solid line in Fig. 4. It is apparent that cleavage of surface-bound prekallikrein by surface-bound α-HF_a occurs very rapidly. As noted in the figure legend, a

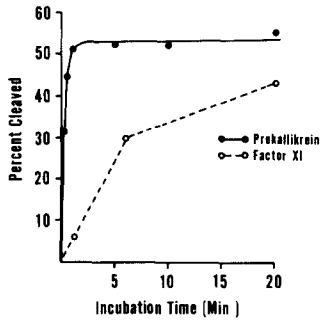

FIG. 4. Cleavage of surface-bound factor XI and prekallikrein by surface-bound α-HF_a. Factor XI: samples of diluted normal plasma containing ^{125}I-factor XI were shaken in glass tubes. After 2 min, the supernates were removed and the tubes washed. 2 ml of buffer was then added to each tube and, after varying incubation times, the surface-bound proteins were eluted with SDS-mercaptoethanol. The samples were analyzed by SDS-polyacrylamide gel electrophoresis for the percent of ^{125}I-factor XI cleaved (dashed line). Prekallikrein: samples of diluted HF-deficient plasma containing ^{125}I-prekallikrein were shaken in glass tubes for 5 min, the supernates removed, and the tubes washed. Samples of diluted prekallikrein-deficient plasma were then added to each tube, followed 20 s later by 2 ml buffer. This buffer was then removed, a fresh 2 ml buffer added, and the tubes incubated for varying times. The surface-bound proteins were eluted with SDS-mercaptoethanol and the cleavage of the ^{125}I-prekallikrein determined by SDS-polyacrylamide gel analysis. Results are shown as the solid line. Note that the use of two different experimental protocols prevent the direct comparison of the two sets of data shown in this figure.

direct comparison of the rates of cleavage of the ^{125}I-prekallikrein and the ^{125}I-factor XI shown in Fig. 4 cannot be made since different experimental procedures were employed.

The Action of β-HF_a on Surface-Bound Factor XI and Prekallikrein. Preliminary experiments were performed to determine the dilution of plasma required to obtain complete binding of HF to glass and generate sufficient β-HF_a in the supernate to yield measurable rates of cleavage of prekallikrein and/or factor XI. The supernate of a 5 min, room temperature, incubation in a glass tube of 20 μl of a $^1/_{25}$ dilution of normal plasma contained <4% (0.0008 μg) native HF and >82% of the β-HF_a formed. This corresponds to 0.0018 μg of β-HF_a. This procedure was used to generate β-HF_a for the experiments described below: Cleavage of surface-bound factor XI by β-HF_a was studied by using ^{125}I-factor XI added to HF-deficient plasma. 20 μl of a $^1/_6$ dilution was allowed to incubate in glass for 5 min with shaking to allow maximal binding of the factor XI to the surface. After removal of the supernate and three buffer washes of 200 μl each, 15 μl of β-HF_a was added to each tube. The tubes were allowed to incubate with shaking for varying times before 50 μl of SDS-mercaptoethanol solution was added to stop the reaction. Cleavage of the ^{125}I-factor XI was assessed by electrophoresis on SDS- polyacrylamide gels. As shown by the dashed line in Fig. 5, no cleavage was observed over a 20 min time period.

An identical experiment was performed with ^{125}I-prekallikrein instead of ^{125}I-factor XI to assess the cleavage of surface-bound prekallikrein by β-HF_a. The results, shown as the solid line in Fig. 5, show a significant cleavage of ^{125}I-prekallikrein by β-HF_a. The prekallikrein cleaved, however, may not all be surface bound. As will be shown below, some of the bound prekallikrein molecules may dissociate from the surface during the activation period and be

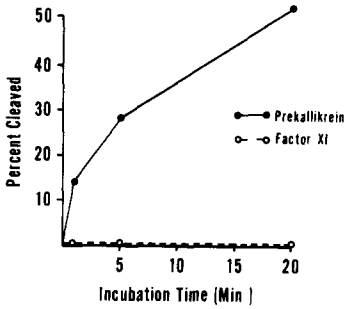

FIG. 5. Cleavage of surface-bound factor XI and prekallikrein by β-HF_a. Samples of diluted HF-deficient plasma containing either ^{125}I-factor XI or ^{125}I-prekallikrein were shaken in glass tubes for 5 min. The supernates were removed and the tubes washed. β-HF_a (see text for details of preparation) was then added to each tube. After varying incubation times, the reactions were stopped and the proteins eluted with SDS-mercaptoethanol. Cleavage of the ^{125}I-factor XI and ^{125}I-prekallikrein was assessed by electrophoresis of the samples on SDS-polyacrylamide gels.

cleaved in solution. Thus we cannot be certain that the cleavage of prekallikrein shown in Fig. 5 is strictly the cleavage of surface-bound prekallikrein or if the cleavage of dissociated molecules in solution is also represented.

The Action of β-HF_a on Factor XI and Prekallikrein in Solution. Possible cleavage in solution of factor XI and prekallikrein by β-HF_a was also investigated. 15 μl of β-HF_a, obtained as noted above, were added to plastic tubes containing 5 μl of a 1/6 dilution of HF-deficient plasma containing either ^{125}I-factor XI or ^{125}I-prekallikrein. The tubes were incubated for varying periods of time and the reactions stopped by the addition of 50 μl of SDS-mercaptoethanol. After SDS polyacrylamide gel analysis of the samples, the percent of radiolabeled protein cleaved was determined; the results are shown in Fig. 6. Prekallikrein in solution could be cleaved by β-HF_a but no cleavage of factor XI was observed.

In a similar experiment, the β-HF_a solution was incubated for 5 min with 1 mg of Sepharose beads containing covalently bound 10 μg of either goat anti-human HF or normal goat gamma globulin before the addition of the ^{125}I-prekallikrein in HF-deficient plasma. Normal cleavage of prekallikrein occurred in the presence of the control beads but no cleavage was seen in the samples incubated with beads containing anti-HF. This experiment confirms that the cleavage of prekallikrein is specifically caused by an active form of HF and not any other proteolytic enzymes which might be present.

Dissociation of HF, Factor XI, Prekallikrein and Kallikrein from a Glass Surface in the Presence of Plasma. Wiggins et al. have recently reported that during contact activation, approximately 60% of the HF, 80% of the factor XI, and 15% of the prekallikrein in plasma became surface bound (6). In conjunction with our studies of the activation of surface-bound factor XI and prekallikrein by the two forms of HF_a, it was necessary to determine the kinetics of elution of these proteins from the surface. The dissociation of surface-bound HF was first studied by using HMWK-deficient plasma to prevent HF activation (1). 20-μl aliquots of a 1/6 dilution of HMWK-deficient plasma containing ^{125}I-HF were incubated in glass tubes for 5 min. The supernates were removed and the tubes, containing those proteins which had become bound to the surface, were

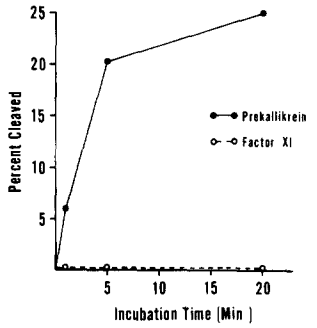

FIG. 6. Cleavage of factor XI and prekallikrein in solution by β-HF_a. Samples of β-HF_a (see text for details of preparation) were added to plastic tubes containing diluted HF-deficient plasma containing either ^{125}I-factor XI or ^{125}I-prekallikrein. After varying incubation times, the reactions were stopped by the addition of SDS-mercaptoethanol and the samples assayed for cleavage of the radiolabeled proteins by electrophoresis on SDS-polyacrylamide gels.

rapidly washed with three successive washes of 200 μl buffer. The dissociation of the ^{125}I-HF from the surface was monitored by adding 200 μl of a $^1/_6$ dilution of HMWK-deficient plasma to each tube, incubating at room temperature with shaking, and removing the supernates at varying times. Since no activation of HF occurs in HMWK-deficient plasma during the time periods used, the appearance of radiolabel in the supernates during the second incubation period represents the dissociation of native ^{125}I-HF from the surface. Approximately half of the ^{125}I-HF added to each tube became surface bound during the initial 5 min incubation. With this amount being considered as 100% bound at the beginning of the elution incubation, the percent remaining bound after various times is shown as the dashed line in the top panel of Fig. 7. To consider the possible influence of HMWK on the binding and/or elution of HF from the surface, the identical experiment was performed using prekallikrein-deficient plasma instead of HMWK-deficient plasma. Those results are shown as the solid line in the top panel of Fig. 7. The data indicate that, under the conditions employed, 25–50% of the HF became dissociated from the surface in 10 min.

Employing the same experimental conditions, the elution of surface-bound factor XI was studied using ^{125}I-factor XI added to HF-deficient plasma. Approximately 65–70% of the ^{125}I-factor XI initially present became bound during the first 5 min incubation period. The results, shown in the center panel of Fig. 7, indicate a very slow rate of dissociation for this molecule.

Prekallikrein elution was determined in a similar manner again using HF-deficient plasma and ^{125}I-prekallikrein. In this case, however, only 10–15% of the radiolabeled protein became initially bound and that underwent a rapid and extensive elution during the second incubation (Fig. 7, bottom panel). When normal, rather than HF-deficient plasma was used, an almost identical elution profile was obtained. Since most of the prekallikrein is cleaved to kallikrein under these conditions, an approximately equal dissociation rate is obtained for the activated enzyme.

It should be noted that in all of the experiments just described, the amount of a specific protein found dissociated from the surface at any given time will vary with both the volume and protein concentration of the fluid phase.

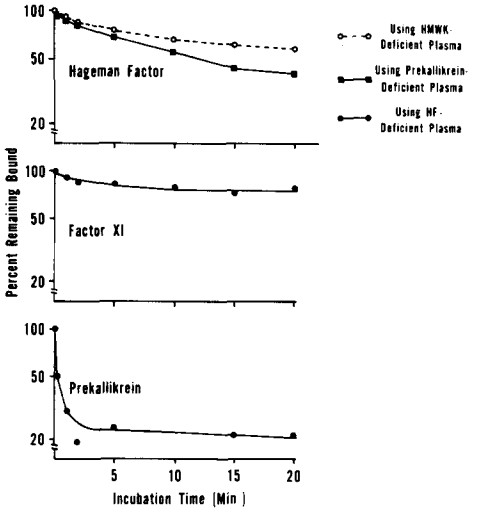

FIG. 7. The elution of surface-bound HF, factor XI, and prekallikrein with plasma. 20-μl samples of a $^1/_6$ dilution of HMWK-deficient and prekallikrein-deficient plasma containing ^{125}I-HF, HF-deficient plasma containing ^{125}I-factor XI, and HF-deficient plasma containing ^{125}I-prekallikrein were incubated in glass tubes at room temperature for 5 min. The supernates were then removed and the tubes rapidly washed with three washes of 200 μl buffer. 200 μl of a $^1/_6$ dilution of the same plasma used for the first incubation was then added to each tube and incubation continued at room temperature. Elution of the bound radiolabeled proteins was followed by removing the supernates at various times and counting the radioactivity remaining in the tubes.

Dissociation of kallikrein from the surface was also studied using immunologic techniques. 2 ml of normal human plasma was mixed with 20 mg of kaolin in a plastic tube for 15 min at 22°C to allow maximum binding and activation of the prekallikrein. The mixture was centrifuged for 3 min and the supernate removed. The kaolin pellet was resuspended and washed for 30 s with 200 μl buffer and centrifuged for 2 min to reform a pellet. This washing was rapidly repeated a total of three times. All washes were saved for later analysis. The washed kaolin pellet was then subjected to four successive elutions each consisting of a 30 s incubation with 200 μl of prekallikrein-deficient plasma diluted $^1/_2$ followed by a 2 min centrifugation. Each elution supernate was removed and analyzed, along with the washes, by radial immunodiffusion for the amount of kallikrein present. Of the kallikrein remaining bound to the kaolin after the three buffer washes, 84% was dissociated and recovered in the four brief incubations with plasma. This agrees favorably with the rapid dissociation of ^{125}I-kallikrein reported above.

Discussion

The data reported here show that the two forms of active HF produced by contact activation of plasma show differences in their ability to activate prekallikrein and factor XI. These differences depend, in part, upon whether the enzyme and the substrate are surface bound or in solution. It was found that surface-bound α-HF_a can cleave both prekallikrein and factor XI when they are surface bound. β-HF_a, in solution, was found to cleave prekallikrein in solution or on a surface but had no demonstrable activity on factor XI

whether bound or in solution. These activities, along with the structural characteristics and derivation of the activated forms are summarized in Fig. 1.

The finding that surface-bound α-HF_a can activate both substrates when they also are surface bound implies that either the enzyme and the substrates initially bind to the surface in adjacent positions or that at least one of the reactants can change its location on the surface. The latter is an unlikely explanation for the experiment as presented since the presence of a large volume of buffer during the incubation period would result in at least a 600-fold dilution of any protein eluting from the surface, thus making it unlikely that an eluted molecule would bind again.

When the cleavage of the two substrates in solution by β-HF_a was investigated, the β-HF_a was found to have selective activity; that is, prekallikrein was cleaved while factor XI was not (Fig. 6). Even when the substrates were surface bound, β-HF_a was found capable of cleaving only prekallikrein (Fig. 5). It should be stressed that the observed failure of any factor XI to be cleaved by β-HF_a is contingent upon the conditions of the experiment. In other systems, with greater concentrations of reactants, it might well be possible to demonstrate some cleavage of factor XI by β-HF_a. The present study does show, however, that the very weak procoagulant activity often reported for the lower molecular weight form of HF_a as compared to the higher molecular weight form(s), is caused by its poor reactivity with factor XI. By contrast, this molecule is an excellent activator of prekallikrein. The reasons for the ability of β-HF_a to react readily with prekallikrein but not with factor XI are unclear.

Wiggins et al. (6) have recently found that 80% of the factor XI in plasma, after contact activation, is surface bound, while only 20% of the kallikrein is found on the surface. Our studies support these findings. The elution studies indicate that the association between prekallikrein and the surface is weak; of the molecules surface bound at a given time (zero time), almost 80% can be eluted within 5 min. By contrast, factor XI was found to be firmly attached to the surface.

The observation that factor XI must be surface bound to be efficiently cleaved and that once cleaved the fragments remain surface bound (6) strongly suggest that the first two steps of the intrinsic coagulation system are localized events. The kinin-generating and fibrinolytic systems, however, can be initiated either locally by the cleavage of prekallikrein by surface-bound α-HF_a or in the fluid phase by β-HF_a. In either case, kallikrein, once formed, exists primarily in solution. The data presented here suggest that in vivo the activities of the kinin-forming and fibrinolytic systems would disseminate rapidly from the initial locus of activation, while those of the contact phase of the intrinsic clotting system would remain localized. The significance of these observations in vivo will be appreciated in further experiments.

Summary

The ability of the two forms of activated Hageman factor (HF_a) produced during contact activation of plasma to activate prekallikrein and factor XI was studied. α-HF_a, defined as an 80,000 mol wt two-chain enzyme which remains bound to the surface was capable of cleaving surface-bound prekallikrein and factor XI. β-HF_a, a 28,000 mol wt single chain molecule, released from the surface during contact activation was able to cleave prekallikrein but showed

no activity on factor XI. Cleavage of prekallikrein by β-HF_a occurred irrespective of whether the substrate was surface-bound or in solution. Cleavage of factor XI occurred only when it was surface bound and only the α-form of HF_a was capable of this proteolytic action. Factor XI was found to remain bound to the surface while prekallikrein and kallikrein rapidly dissociated from the surface into the supernate. These findings suggest that the initiation of intrinsic coagulation through the activation of factor XI is a localized event occurring at the site of contact activation and is the result of the action of α-HF_a. By contrast, kinin generation and fibrinolysis resulting from the formation of kallikrein can be initiated either at the site of contact activation, by α-HF_a action, or throughout the plasma, by β-HF_a; further dissemination of these activities is assured by the rapid dissociation of kallikrein itself from the surface.

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