DISSEMINATION OF CONTACT ACTIVATION IN PLASMA BY PLASMA KALLIKREIN*

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Previous studies have provided evidence that during contact activation of plasma, Hageman factor (HF, clotting Factor XII)[1] binds to the negatively charged surface along with high molecular weight kininogen (HMWK). The HMWK circulates complexed at least in part with prekallikrein (PK) (1) or clotting Factor XI (2) and is instrumental in bringing both PK and clotting Factor XI to the surface (3). The approximation of HF with PK leads to limited proteolytic cleavage and activation of both molecules, each serving as substrate for the other (4–8). Although it is not demonstrated unequivocally, the initiating event in the activation of these two zymogen enzymes may result from the interaction of the two molecules acting as "active zymogens": single-chain, zymogen HF and PK react with diisopropylphosphofluoridate with second-order rate constants of $0.36M^{-1} min^{-1}$ and $0.58M^{-1} min^{-1}$ comparable in value to that of trypsinogen (9). In addition, zymogen HF is capable of slowly cleaving adjacent HF molecules on a negatively charged surface, leading to autoactivation (10).

It is not clear whether activation of HF on the surface results entirely from an assembly of zymogen HF and PK complexed with HMWK on a surface or whether a factor exists in solution that could disseminate the reaction rapidly. In the present paper we have investigated this question by employing radiolabeled proteins and following the propagation of the reaction both on the surface and in solution. A preliminary report has been presented (11).

Materials and Methods

Protein Reagents. The methods of purification of HF, PK, HMWK, and Factor XI from human plasma have been reported previously (12, 13). The purified proteins were radiolabeled with ^{131}I or ^{125}I by the chloramine T method (14). Specific activity varied from 2.4 to 12.4 $\mu Ci/ \mu g$ protein.

Buffer. Tris-buffered saline (TBS) consisting of 0.01 M Tris with 0.15 M NaCl pH 7.4 was used throughout these studies.

Kaolin. (J. T. Baker, Phillipsburgh, N. J.) was washed and suspended in TBS at 20 mg/ml. The suspension was maintained in aliquots at −20°C until used.

Plasma. Normal human plasma was obtained from 19 individuals using 1/6 volume acid-

[1] *Abbreviations used in this paper:* HF, Hageman factor; HMWK, high molecular weight kininogen; 2ME, 2-mercaptoethanol; PAGE, polyacrylamide gel electrophoresis; PK, prekallikrein; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline.

citrate dextrose as an anticoagulant. The pooled plasma was frozen in aliquots and stored at −70°C until used. Plasma deficient in HF was generously donated by Mrs. Gunda Hiatt.

Procedure of Separating Surface-bound Recipient HF from HF on a "Generating" Surface.   To determine if HF activation can take place at a distance from the position where the generation of HF activity is proceding, ^{125}I-HF and ^{131}I-HF were placed on separate kaolin particles as follows (Fig. 1): ^{131}I-HF was added to fresh normal human plasma at a ratio of 0.05 μg/0.1 ml and cooled to 4°C before being diluted 1/2 with TBS. 30 μl of this was then added to 40 μl kaolin suspension (800 μg) at 4°C and rapidly mixed for 1 min before rapid sedimentation in a Beckman Microfuge (Beckman Instruments, Fullerton, Calif.) and washed three times in 200 μl TBS at 4°C. As recipient HF on a surface, ^{125}I-HF was added to PK-deficient (Fletcher trait) plasma and diluted 1/2 in TBS before being exposed to kaolin in a manner identical to that above. The two washed sets of kaolin particles were resuspended in 0.1 ml TBS at 4°C, combined and rapidly mixed, and warmed to 37°C, and incubated with agitation for the times noted below. At the designated time, 50 μl of 4% sodium dodecyl sulfate (SDS) containing 2% 2-mercaptoethanol (2ME) were added and the samples placed at 100°C for 3 min. Cleavage of the radiolabeled HF was then examined in polyacrylamide gel electrophoresis (SDS-PAGE). To determine if the type of iodine isotope in any way influenced the experiment, the ^{125}I-HF was added to normal plasma and the ^{131}I-HF was added to PK-deficient plasma and tested as above. In each case, 32 ± 5% of the radiolabeled HF was bound to the kaolin particles after washing. 43% of the HF on the particles exposed to normal plasma had undergone cleavage into 52,000- and 28,000-mol wt fragments (in the presence of 2ME) whereas <5% of the HF on particles exposed to PK-deficient plasma was cleaved. Greater than 90% of the HF remained surface bound after incubation at 37°C. Greater than 70% of the isotopically labeled HF was recovered in the acrylamide gels.

Preparation of Antibodies.   Antisera were obtained from goats immunized with purified human HF, PK, and plasminogen. Goat antibodies to the light chain of HMWK were obtained from Dr. D. Kerbiriou and Dr. J. H. Griffin, Scripps Clinic and Research Foundation, La Jolla, Calif. The antisera were twice absorbed with 25 mg/ml kaolin and the γ-globulin fractions subsequently precipitated with 40% ammonium sulfate. After dialysis to remove the salt, the antibodies were passed over Sepharose beads to which were coupled the corresponding antigens. After thorough washing of the beads, immunopurified antibodies were eluted with 5 M guanidine and dialyzed against phosphate-buffered saline, pH 7.0. The immunopurified antibodies were then coupled to Sepharose 4B beads to a concentration of 5 mg protein/g beads.
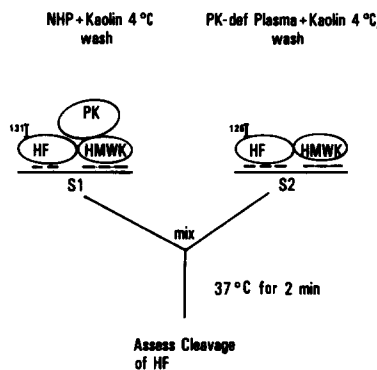

Fig. 1. Experimental protocol for the dissemination of activation of HF. The full generating system of contact activation with ^{131}I-HF is shown on the left (site 1 or S1) and the recipient ^{125}I-HF is on the right (site 2 or S2). The negatively charged surface (kaolin particles) is represented by the horizontal lines. After incubation at 4°C to effect binding the two sets of particles were washed and then mixed. Following incubation with shaking at 37°C for intervals up to 120–140 s, SDS containing 2-ME at 100°C was added to stop the reaction and cleavage of the radioisotopically labeled HF molecules were assayed in SDS-PAGE. Reversal of the ^{125}I- and ^{131}I-labeled HF did not alter the results.

Results

Propagation of the Cleavage of HF on a Surface. Radiolabeled ^{131}I-HF was added to normal human plasma and exposed to a first set of kaolin particles at 4°C, and ^{125}I-HF was added to PK-deficient plasma at 4°C and exposed to a second set of kaolin particles as noted in Materials and Methods. After washing, the two sets of particles were mixed and incubated at 37°C for 10, 30, 60, and 140 s at which time the reaction was stopped by addition of SDS-2ME at 100°C. SDS-PAGE revealed cleavage of both ^{125}I-HF and ^{131}I-HF as noted in Fig. 2. The cleavage pattern of HF was identical for the two isotopically labeled proteins, with heavy chains of 52,000 and light chains of 28,000 appearing as cleavage progressed. When the two sets of kaolin particles were incubated separately, i.e., without mixing, cleavage of the HF occurred to the same extent in particles exposed to normal plasma, but in the 140-s time period, no cleavage of HF occurred in particles exposed to PK-deficient plasma. In the absence of reducing agent, the 80,000 mol wt failed to dissociate into heavy and light chains. When the addition of isotopically labeled HF preparations to the normal or PK-deficient plasmas was reversed, no difference in the extent of cleavage of the HF was observed at each time point. Thus the capacity to cleave HF into its 52,000- and 28,000-mol wt subunits was transferred from one set of kaolin, exposed to normal plasma, to the HF bound to a second set of kaolin particles, exposed to PK-deficient plasma.

To test the possibility that the active material that cleaved HF on the second set of kaolin particles was generated as a result of the activation of contact systems proteins, HF-deficient plasma (without added radiolabeled HF) was substituted for normal plasma on the first set of kaolin particles. The two sets of particles were mixed and incubated at 37°C for 30 s and the ^{125}I-HF (on the second set of kaolin particles) was assessed for cleavage in SDS-PAGE. No cleavage was observed, suggesting that an intact contact system was required. However, when the incubation period employing HF-deficient plasma on the first set of particles was extended to 120 s, cleavage of the ^{125}I-HF on the second set of particles occurred up to two-thirds of that when the first set of particles was exposed to normal plasma (Fig. 3).

The above experiments suggested that a soluble factor was generated when the normal plasma contacted kaolin. To test this possibility the supernate of the kaolin particles that had been contacted with normal plasma and incubated at 37°C for 140 s was obtained and added separately to the ^{125}I-HF on the second set of kaolin
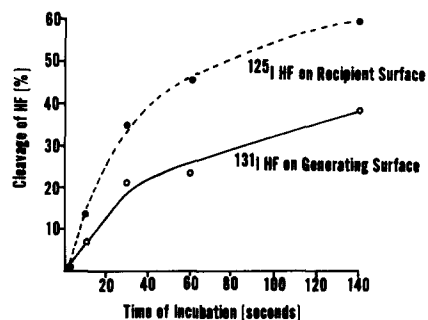

Fig. 2. Cleavage of radiolabeled HF on generating ^{131}I-HF (site 1) and recipient ^{125}I-HF (site 2) surfaces after mixing of the particles at 37°C for the times indicated (see Fig. 1). Cleavage was measured by loss of radiolabeled protein at the native MW or appearance of the 52,000-mol wt cleavage fragment.
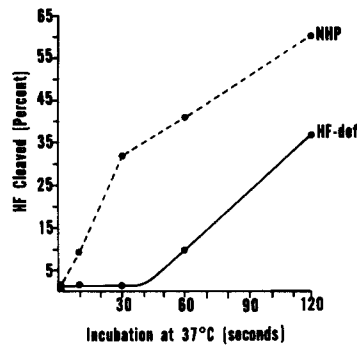
Fig. 3. Cleavage of radiolabeled HF on recipient (site 2) surfaces after exposure to kaolin particles treated with normal (NHP) or HF-deficient (def) plasma on the generating (site 1) surface. The conditions are identical to those of Figs. 1 and 2.
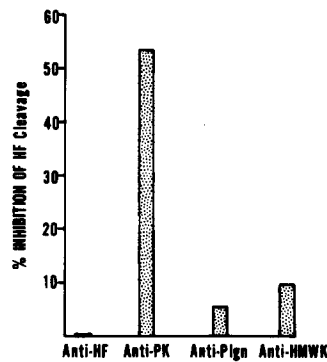

Fig. 4. Inhibition of the soluble active principal generated on the first surface, by antibodies to proteins of the contact system. The supernate of incubation on site 1 was mixed with purified antibodies as shown and immediately placed on recipient, site 2 kaolin particles bearing ^{125}I-HF. Cleavage was assessed in SDS-PAGE after a 120-s incubation at 37°C.

particles that were exposed to PK-deficient plasma. Cleavage of the radiolabeled HF took place to the same extent as when the two sets of particles were mixed as noted above.

Identification of the Soluble Factor Responsible for Cleavage of HF on the Kaolin Particles. Since the contact system appeared to play a role in generation of the soluble factor, antibodies to HF, PK, plasminogen, and HMWK, insolubilized on Sepharose 4B, were added to the supernatant fluid that was obtained from the kaolin particles (800 μg) exposed to normal plasma (30 μl, diluted 1/2) and incubated at 37°C for 120 s. The results, shown in Fig. 4, revealed that only antibodies to PK inhibited significantly the capacity of the soluble factor to cleave surface-bound, radiolabeled HF. The data suggested that kallikrein was responsible for dissemination of the contact activation of HF.

The Binding and Extent of Cleavage of HF, PK, HMWK, and Plasminogen in Plasma during Contact Activation. From the foregoing data, it appeared that prekallikrein is rapidly converted to kallikrein upon surface contact and that kallikrein rapidly appears in the fluid phase. Because the experiments noted above required washing of the kaolin before assessment of the dissemination, it was important to obtain information on the

rate of cleavage, activation, and appearance in fluid phase of activated components during surface contact. Accordingly, studies were conducted to measure the rate of cleavage of HF, PK, HMWK, and plasminogen during contact of plasma with kaolin, and on the rate of appearance of cleavage fragments in the supernatant fluid.

[125]I-Labeled HF, PK, HMWK, or plasminogen were added to 1/2 diluted normal human plasma at 37°C. 40 μl was then exposed to 800 μg kaolin at 37°C with constant agitation. Samples were removed after 0-, 20-, 40-, and 60-s incubation at 37°C, and the kaolin was separated by rapid sedimentation. The supernate was immediately removed from the kaolin by aspiration, and the kaolin pellet was washed by resuspending in 200 μl saline and resedimenting. SDS-2ME at 100°C was added immediately to the initial supernate and, after washing, to the kaolin pellet. 15 s was required for the initial separation and an additional 20 s for the washing procedure. The amount of the radiolabeled proteins bound to the kaolin or in the original and washing supernate were measured, and proteins of both the original supernatants and the kaolin pellet were subjected to SDS-PAGE in the presence of 2ME. The data, showing representative figures from one of three experiments, are shown in Table I. Because 35 s were required to sediment and wash the kaolin, there was appreciable binding of the components at the initial time point (designated 0 s). It is of note that although the amounts of HF and HMWK that bound increased over the 60-s period, the total binding of PK failed to change significantly. When HF-deficient plasma was substituted for normal plasma, the binding of the proteins was not different from that occurring in normal plasma.

To determine if the behavior of the radiolabeled proteins was representative of the population as a whole, unlabeled HF, PK, and HMWK in the plasma and supernate, after exposure to kaolin as noted above, were assayed by quantitative immunodiffusion to measure the concentration of the proteins at the various time points. Changes in the concentration of radiolabeled proteins were closely paralleled by the concentration of individual proteins. In addition, a single study was performed using ^{131}I-HF together with ^{125}I-PK in a paired-label experiment in normal plasma, identical to the single protein experiments noted above in which either radiolabeled protein was examined individually. Binding and cleavage (see below) of the two proteins occurred

TABLE I

Binding of ^{125}I-HF, PK, and HMWK to Kaolin during Incubation in Normal Plasma

	Binding to kaolin*			
	0 s	20 s	40 s	60 s
	%			
^{125}I-HF	20.1	29.3	34.5	31.3
^{125}I-PK	12.0	11.4	14.6	9.6
^{125}I-HMWK	40.4	55.3	64.7	58.9

* ^{125}I-Proteins were added to 1/2 diluted normal human plasma at 37°C (total volume 30 μl). After 15 min 800 μg kaolin in 40 μl was added with shaking and at the time intervals shown, the kaolin was rapidly sedimented and washed. Time elapsed for sedimentation and washing was 37 s. The amount bound to the kaolin was that remaining after washing. Less than 15% of added ^{125}I-proteins appeared in the wash.

at a rate closely similar to that when single isotopically labeled proteins were employed.

Cleavage of each radiolabeled protein was then assessed in the supernatant fluid (Fig. 5) and on the washed kaolin (Fig. 6). The data of one of three experiments are shown. In experiments using normal plasma, 10% of the HF in the supernate was cleaved at the initial time and this failed to increase during the 60-s incubation. The appearance of cleaved ^{125}I-PK in the supernate was rapid as noted in Fig. 5, reaching a value of 72% by 40 s. Similarly, cleaved HMWK in the supernate appeared rapidly, with ~86% being cleaved by 20 s.

On the kaolin surface, the amount of ^{125}I-HF cleaved at the initial time point was 21%, and this rose to 65% by 60 s as shown in Fig. 6. The majority of the ^{125}I-PK and HMWK were cleaved at the initial time point. It should be emphasized that ~35 s elapsed between mixing and addition of hot SDS to the washed kaolin. Cleavage of HF, PK, and HMWK occurred within the disulfide loop of the polypeptide chain and yielded the typical heavy and light chains of each protein upon reduction with 2ME.

Simultaneous studies employing HF-deficient plasma rather than normal plasma revealed <10% cleavage of radiolabeled HF, PK, and HMWK upon contact with kaolin in both supernate and on the surface over the 60-s period. The amount of radiolabeled HF (0.01 μg) was insufficient to generate contact activation in the HF-deficient plasma.

^{125}I-Plasminogen was added to 1/2 diluted normal or C1-inhibitor-deficient plasma and incubated with 800 μg kaolin as noted above. No cleavage of the plasminogen was observed over the 60-s period. ^{125}I-PK added to both plasmas underwent complete
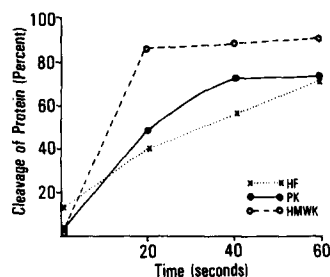

Fig. 5. The appearance of cleaved radiolabeled HF, PK, and HMWK in the supernatant fluid after mixing normal human plasma with kaolin at 37°C. Cleavage was measured by disappearance of radiolabeled protein from its native molecular weight on SDS-PAGE analysis.
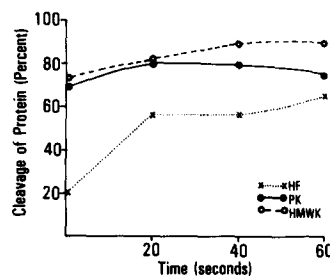

Fig. 6. Cleavage of surface-bound radiolabeled HF, PK, and HMWK. The protocol was the same as in Fig. 5.

cleavage into heavy and light chains in the 60-s period. As positive control, urokinase added to the ^{125}I-plasminogen-normal plasma mixture induced cleavage of the plasminogen.

The Relative Roles of βHF$_a$ in the Supernate and αHF$_a$ on the Surface in the Cleavage of Prekallikrein. Experiments were then performed to determine the relative roles of activated HF either in the supernate or on a negatively charged surface in inducing cleavage of PK. Glass was employed in these experiments to limit the amount of HF bound.

To accomplish this, ^{131}I-HF was added to normal human plasma and ^{125}I-PK was added to HF-deficient plasma. Both plasmas were diluted 1/2 with saline and maintained at 37°C. The ^{131}I-HF-normal plasma (30 μl) was exposed to glass in a series of 12 × 75-mm borosilicate tubes for 60 s with shaking to obtain binding and activation of the HF. The supernates were removed and each was added to 30 μl of HF-deficient plasma containing ^{125}I-PK in plastic tubes and incubated at 37°C for 0–40 s, with samples taken at 10-s intervals to assess cleavage of the ^{125}I-PK by SDS-PAGE. The activated ^{131}I-HF-normal plasma remaining in the bottom of the borosilicate tubes was washed with 200 μl saline, and after aspiration, 30 μl HF-deficient plasma was added for 60 s, followed by 30 μl HF-deficient plasma containing ^{125}I-PK. These tubes were then incubated at 37°C for the same time intervals as the supernatant fluids noted above. In all cases, the reaction was stopped by addition of SDS-2ME at 100°C.

In this way, the relative cleavage of the ^{125}I-PK by the activated HF in the supernatant fluid and on the glass surface could be compared. In addition, the amount of the HF bound to the surface and released into the supernate could be measured, and cleavage of the molecules could be assessed by SDS-PAGE.

The data showed that 11.2 ± 1.8% of the HF in normal plasma was bound to the glass tube after 60 s, representing 0.04 μg. An average of 47% of that HF bound initially was eluted during the 60 s of the second incubation and no additional HF eluted after beginning the second portion of the experiment, i.e., with the addition of ^{125}I-PK in HF-deficient plasma. Thus the amount of activated HF bound to the glass surface (0.021 μg) was constant.

To assess the binding of ^{125}I-PK, in a parallel series of glass tubes, 30 μl ^{131}I-HF in 1/2 diluted normal plasma was first added to the tubes and incubated for 60 s, followed by 30 μl 1/2 diluted HF-deficient plasma for 60 s, and then 30 μl 1/2 diluted HF-deficient plasma containing ^{125}I-PK for 10-s intervals. The amount of ^{125}I-PK bound to the surface in these tubes was 2.1% at 0 time, 5.8% at 20 s, and 12.1% at 40 s. These values are similar to those noted above.

Cleavage of the ^{125}I-PK at each time interval was then assessed in SDS-PAGE, and the amount of ^{125}I-PK cleaved by activated HF in the supernatant fluid or on the surface was compared. The data, shown in Fig. 7, indicate that ^{125}I-PK contacting the surface was cleaved in a time-dependent manner, while no cleavage of the ^{125}I-PK in the presence of the supernatant fluid was detected. Previous experiments, noted above, had shown that cleavage of ^{125}I-PK did not occur when HF-deficient plasma, rather than normal plasma, was employed in the initial incubation.

Of the HF on the surface of the borosilicate tubes >90% was cleaved into disulfide-linked 52,000- and 28,000-mol wt fragments (αHFa) at the initial time period when ^{125}I-PK in HF-deficient plasma was added.
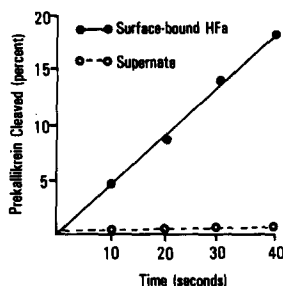
FIG. 7. Cleavage of ^{125}I-PK by HFa bound to a glass surface or in the supernatant fluid.

From the measurements that ~0.02 μg HF (2.5 × 10^{-13} mol) remained surface bound, and only the surface bound HF was able to cleave PK, and from the measurement that 28% of the ^{125}I-PK was cleaved during 1-min contact with the surface (or 0.42 μg or 5 × 10^{-12} mol/min), indicates that 1 molecule of HFa on the surface cleaved ~20 molecules of PK/min.

Discussion

The cleavage and activation of HF and PK occurs when these two molecules are brought together with HMWK on a negatively charged surface. Previous data have indicated that the HMWK acts as a cofactor to bring PK to the negatively charged surface in a position adjacent to HF (3). The PK and HF then interact so as to induce a specific proteolytic cleavage in each molecule with an attendant conversion of the zymogen HF and PK to active enzymes.

In the current study, we have examined the dissemination of this reaction. The data indicate that the burst of activity occurring during contact activation of plasma results in great part from the rapid dissemination of the reaction in fluid phase by kallikrein.

This conclusion was obtained by examining the cleavage of HF on two separate surfaces, one surface in which all molecules of the contact system were present to generate surface-related activity, and a second surface, removed from the first, in which only HF and HMWK were bound. Dissociation of kallikrein from the first surface, presumably from HMWK, was found to occur rapidly, leading to cleavage of HF on the surface at the second, distant surfaces. Cleavage of HF on the second surface occurred as fast as on the first surface, suggesting that much of the cleavage of HF even on the first surface could result from the fluid-phase dissemination of the reaction. The conclusion that the disseminating agent was kallikrein, rather than some other enzyme, was obtained from studies in which antibodies to kallikrein, but not other proteins, inhibited the dissemination of the HF-cleaving activity. In addition, plasma deficient in PK failed to generate the active principle in fluid phase. The ability of the dissociated kallikrein to cleave surface-bound HF molecules attests to its retaining enzymatic activity. The relatively small amount of binding of kallikrein to the surface and the rapid dissociation have previously been shown in this laboratory (3, 15). An association constant of 3.4 × 10^7 M^{-1} has recently been attributed to the reaction between PK and surface-bound HMWK (16). A model of the dissemination of contact activation by kallikrein is shown in Fig. 8 in which the contact activation of plasma is divided into three phases.
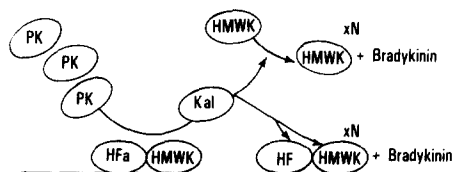
Fig. 8. Schematic diagram of the proliferative and disseminating phases (Summary) of plasma. HF in the generating unit (phase 1) of HMWK and HF at the left has already undergone activation (HFa). Phase 2: Multiple molecules of PK interact with the HFa and HMWK on the surface and are converted to kallikrein (kal). Phase 3: Kallikrein then disseminates the reaction, acting upon both HF and HMWK on the surface and HMWK in fluid phase. The multiple reactions of this latter nature are designated xN in the diagram.

The data suggest that a single surface-bound pair of HFa and HMWK molecules can activate many molecules of PK which, in the form of kallikrein, dissociate, and cleave HF and HMWK, at a distance. With prolonged incubation of plasma with a negatively charged surface only ~10% of the PK or kallikrein was found on the surface at any one time, while increasing amounts of HMWK and HF became surface bound. Upon examination of the radiolabeled PK by SDS-PAGE, ~50% of the molecules in the supernate and 80% on the surface were cleaved within 20 s. Cleavage of the PK in the fluid phase could occur by two means: activated HF on the surface could cleave PK in the adjacent position, and/or activated HF in the supernate could cleave PK. Experiments performed to differentiate these two possibilities showed that with a limited amount of surface at least 17-fold more kallikrein was generated by HFa on the surface than in the supernatant solution in a 40-s period. β-HFa in the supernate did not cleave PK in the short time of incubation (40 s) and under the conditions of the experiment. That β-HFa with longer exposure, or in greater concentration, can cleave and activate isolated PK, has been documented (17–20).

Calculations of the number of PK molecules that interacted with activated HF on the surface showed that an average of 20 PK molecules were activated per minute for each active surface-bound HF molecule. This figure represented a minimal number because the concentration of components was maintained at a minimum to reduce the formation of β-HFa that would dissociate from the surface. The rapid dissociation is a likely explanation for the low percent binding of PK to the surface (presumably to HMWK) as was also found previously (3).

Studies of ^{125}I-HMWK revealed that this molecule rapidly and firmly binds to the surface, and, unlike kallikrein or β-HFa, the ^{125}I-HMWK remained surface bound. Intensive washing failed to dissociate the HMWK. Both the surface bound and fluid-phase HMWK underwent rapid cleavage during contact activation, with up to 85% of the HMWK being cleaved within 20 s in the fluid phase in some experiments. Studies in which PK or HF-deficient plasmas were substituted for normal plasma indicated that contact activation was required for cleavage of the ^{125}I-HMWK in both fluid phase and on the surface. Recent studies of Kerbiriou and Griffin (13) have revealed that the rapid cleavage of the HMWK, under conditions similar to those employed herein, is associated with release of bradykinin.

These studies indicate that kallikrein, formed by the interaction of its zymogen, PK, with HMWK and HF on a surface, rapidly dissociates and acts upon HF and HMWK at sites removed from the site of its generation. Because a minimum of 20 PK molecules react with each HFa on the surface per minute, it appears that the

repeated conversion of PK to kallikrein on a generating surface contributes in a major way to the explosive activation of the contact system. This burst of activity by kallikrein contributes to the activation of neighboring, surface-bound HF molecules and of both fluid-phase and surface-bound HMWK.

We did not observe cleavage of ^{125}I-plasminogen during contact activation of normal or Cl-inhibitor-deficient plasma, whereas urokinase was capable of effecting cleavage. That kallikrein was generated was shown by complete cleavage of radiolabeled PK into heavy and light chains during the reaction. The reason for a lack of cleavage of plasminogen in plasma during contact activation is unclear. Studies with isolated proteins have indicated that kallikrein and Factor XI are capable of activating plasminogen (21, 22). Nevertheless, inhibitors in plasma and, possibly the low quantity of kallikrein in plasma may account for the lack of activity.

Summary

The dissemination of contact activation of plasma was examined by measuring the cleavage of Hageman factor (HF) molecules on two separate sets of kaolin particles, one of which contained all of the components of the contact activation system, HF, prekallikrein (PK) and high molecular weight kininogen (HMWK) in whole normal plasma, and the second set of particles containing only HF and HMWK, being prepared with PK-deficient plasma. After mixing of the particles, cleavage of HF on the second set of particles occurred at a rate similar to that occurring on the first set of particles. This indicated that rapid dissemination and burst of activity of the contact reaction takes place in fluid phase. A supernatant factor, responsible for the dissemination of the contact reaction, was identified as kallikrein.

A rapid appearance of cleaved PK (kallikrein) and HMWK on both the kaolin surface and in the supernate was observed. Within 40 s, >70-80% of the PK and HMWK in the supernate was cleaved. On the surface, ~70% of each radiolabeled protein was cleaved at the earliest measurement. Cleavage of PK by activated HF occurred at least 17 times faster on the surface than in the fluid phase, as virtually no cleavage of PK occurred in fluid phase. Each molecule of surface-bound, activated HF was calculated to cleave at a minimum, 20 molecules of PK per minute.

It is concluded that the contact activation of plasma may be divided into three phases: (a) the reciprocal activation of a few molecules of zymogen HF and PK on the surface, with HMWK acting as cofactor to bring these molecules into apposition; (b) the rapid release of kallikrein into the fluid phase and the continued conversion of PK to kallikrein by each surface-bound molecule of activated HF; and (c) the activation by fluid-phase kallikrein of multiple surface-bound HF molecules, and the cleavage of multiple molecules of HMWK both in fluid phase and on the surface by the soluble kallikrein. The evidence suggests that steps b and c account for a great majority of the generation of contact activation of plasma.

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