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BINDING AND DISSOCIATION OF HAGEMAN FACTOR, PREKALLIKREIN AND HIGH MOLECULAR WEIGHT KININOGEN IN HUMAN PLASMA DURING CONTACT ACTIVATION

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Abstract—A kaolin pellet was incubated with human plasma at room temperature and immediately washed to remove all unbound proteins. Whereas in normal plasma, 154 mU(PPAN) of kallikrein was bound to the kaolin surface, in Hageman factor (HF)-deficient plasma or normal plasma preincubated with polybrene the surface-bound kallikrein was undetected, while a trace of prekallikrein was bound to the kaolin surface. Dissociation of kinin and kallikrein from the kaolin surface occurs during varying periods of incubation of the kaolin suspension alone. The dissociation of the surface-bound kallikrein revealed two phases in a 15 min incubation: the first phase of dissociation which rapidly progressed until the kinin liberation reached a plateau was followed by the second phase where the kallikrein was slowly dissociated and kinin was no longer liberated. Treatment of the kaolin suspension with trypsin, plasmin or plasma kallikrein enhanced the kinin liberation and dissociation of kallikrein from the kaolin surface. Kallikrein–kinin-free high molecular weight (HMW) kininogen–activated HF complex, kallikrein–kinin-free HMW kininogen complex, kallikrein, kinin-free HMW kininogen and two activated HFs were found on Sephacryl S-300 and Sephadex G-100 gel filtrations of the supernatant obtained after a 60 min incubation of the kaolin suspension alone. The ternary complex of kallikrein, kinin-free HMW kininogen and activated HF suggests the presence of prekallikrein–HMW kininogen–HF complex on the kaolin surface.

The exposure of normal human plasma to negatively charged surfaces results in coagulation, fibrinolysis and kinin formation. The binding and activation of Hageman factor (HF) is necessary for the subsequent activation of each of these systems. Recent studies have demonstrated that prekallikrein and high mol. wt (HMW) kininogen are important in the activation of HF. The abnormal rate of activation of these three systems in Fletcher trait plasma [1] was attributed to a deficiency of prekallikrein [2, 3]. Thus, Fletcher factor has been identified as the plasma prekallikrein [4, 5] and kallikrein was shown to play a critical role in the activation of HF [5, 6]. Another factor required for the efficient activation of the HF-dependent reactions was found using plasma of patients named Fitzgerald [7], Williams [8], Flaujeac [9] and Washington [10] who have been shown to lack HMW kininogen. The clotting, fibrinolytic and kinin-forming activities in plasma deficient in HMW kininogen are even more abnormal than in prekallikrein deficiency. HMW kininogen accelerates the activation of prekallikrein and factor XI by surface-bound HF by at least 10-fold, as well as the cleavage of surface-bound HF by kallikrein [11]. HMW kininogen also enhances the function of surface-bound HF without affecting binding of HF to the surface [12] and the ability of HF fragments to activate prekallikrein in solution [13]. Recently Griffin has reported 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; the surface-bound HF was 500 times more susceptible than soluble HF to proteolytic activation by kallikrein in the presence of HMW kininogen [14]. Mandel *et al.* have demonstrated that prekallikrein and HMW kininogen circulate in plasma as a non-covalently bound complex [15]. Wiggins *et al.* have described that HMW kininogen is essential for normal binding of prekallikrein on kaolin surface and that 80 per cent of the kallikrein resulting from the activation of surface-bound prekallikrein was found in solution [16]. Griffin and Cochrane have reported a working hypothesis for the mechanism of contact activation of HF-dependent reactions in which HMW kininogen and HF form a complex on kaolin [11]. These reports suggest that the complex of prekallikrein and HMW kininogen may be absorbed together on an activating surface and be linked with surface-bound HF during surface activation, resulting in the formation of a surface-bound complex of HF, prekallikrein and HMW kininogen which places HF in a more favourable conformation to be cleaved by kallikrein and which places prekallikrein in a more favourable conformation to be cleaved by activated HF. The author found a ternary complex of kallikrein, kinin-free HMW kininogen and activated HF, which suggests the presence of prekallikrein–HMW kininogen–HF complex, dissociated from kaolin surface incubated with normal plasma. The present paper also describes the interaction

among the surface-bound prekallikrein, HMW kininogen and HF, and the dissociation of the ternary complex from the kaolin surface.

MATERIALS AND METHODS

Plasma. Human venous blood was collected in 0.1 vol. of 3.1% sodium citrate with siliconized needles and plastic syringes and centrifuged at 4°. The freshly separated plasma from 3 normal healthy adults was pooled and used for the experiment. Plasma deficient in HF was obtained from DADE Division American Hospital Supply Corporation, Miami, FL, U.S.A.

Contact activation. An aliquot of freshly pooled plasma or HF-deficient plasma was incubated with kaolin (5 mg/ml plasma) in a plastic tube at room temperature for 2 min to allow contact activation of plasma. The mixture was centrifuged for 2 min and the supernatant was removed. The kaolin pellet was resuspended, washed twice for 20 sec with cold 0.05 M Tris buffer (pH 7.8) containing 0.15 M NaCl to remove all nonbound proteins and resuspended in the buffer to the original volume. Plasma was not diluted for the experiment in order to approximate the conditions under which contact activation probably occurs *in vivo*, although incomplete activation of HF was obtained by the activation procedure mentioned above. In order to obtain complete activation of HF, it is necessary to dilute the plasma 1/25 as described by Revak *et al.* [17].

Preparation of kallikrein. Plasma kallikrein partially purified through column chromatography of DEAE cellulose and DEAE-Sephadex A-50 described in the previous paper [18] was subjected to affinity chromatography on a Trasylol-Sepharose 4B according to the method of Cuatrecasas *et al.* [19] and Oza *et al.* [20].

Preparation of prekallikrein. A partially purified preparation of prekallikrein was prepared by the method of Burrowes *et al.* [21] with a slight modification. Twenty millilitres of non-contacted human plasma were subjected to column chromàtography on a 2.8 × 44 cm column of QAE-Sephadex A-50 which was equilibrated with 0.1 M Tris–HCl buffer (pH 8.0). Fractions containing prekallikrein were pooled and concentrated. Aliquots of the concentrated prekallikrein were subjected to gel filtration on a 3.5 × 45 cm column of Sephadex G-200 which was equilibrated with 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl. Almost all of the IgG and all the factor XI could be eliminated as described by Burrowes *et al.* [21]. The pooled fractions of prekallikrein were dialyzed against 0.015 M phosphate buffer (pH 6.5) and applied to a 1.5 × 50 cm column of CM-Sephadex C-50 equilibrated at pH 6.5. A gradient of NaCl was developed in the starting buffer [22]. Prekallikrein activation was performed by adding 5 μg of trypsin to the effluent, incubating for 30 min at 37°, and blocking the trypsin with 20 μg of lima bean trypsin inhibitor (LBTI). The kallikrein activity was measured by BAEe hydrolysis. The specific activity was 0.2 μmoles BAEe hydrolysis/min/mg.

Preparation of prekallikrein–high mol. wt kininogen complex. Partially purified prekallikrein–HMW kininogen complex was prepared by gel filtration and Pevicon-block electrophoresis. Fifteen millilitres of non-contacted human plasma were fractionated on a 3.5 × 50 cm of Sephadex G-200 which was equilibrated with 0.01 M Tris–HCl buffer (pH 7.0) made 0.15 M in NaCl, 10^{-3} M in EDTA, 5×10^{-4} in DFP and 1 mg hexadimethrine bromide/liter. The fractions containing prekallikrein–HMW kininogen complex were pooled, concentrated to 5 ml by ultrafiltration, dialyzed and subjected to electrophoresis in Pevicon. Electrophoresis was perfomed with 0.05 μ barbital buffer (pH 8.6) at 23 mA (260 V) at 4° for 35 hr using a plastic frame (46 × 6.5 × 1.5 cm). The block was sectioned into 1-cm segments and protein was recovered by centrifugation. The supernatant was dialyzed against phosphate buffered saline to remove the barbital buffer.

Preparation of kininogen. Kininogen substrate was prepared as described previously [18].

Assay of kallikrein. Kallikrein activity was measured by cleavage of synthetic substrates and kinin-forming activity. The cleavage of benzoyl-prolyl-phenylalanyl-arginine-*p*-nitroanilide (PPAN) was estimated by the method of Claeson *et al.* [23]. The absorbance at 405 nm was determined in a spectrophotometer. In experiments with kaolin-activated samples, the absorbance at 405 nm was read against buffer treated with an appropriate amount of kaolin as a blank. Prekallikrein on the kaolin surface was activated to kallikrein with 10 μg of trypsin for 15 min at 37°. The trypsin added was blocked an excess amount of LBTI. Results were expressed as milliunits per ml of plasma. The amount of enzyme giving cleavage of 1 μmole PPAN per min at 37° was taken as 1 unit (U). The hydrolysis of benzoyl-arginine-ethyl ester (BAEe) was also measured by the spectrophotometric method of Schwert and Takenaka [24] for kallikrein activity in eluates from Sephacryl S-300 or Sephadex G-100.

Assay of activated HF. The activity of activated HF was determined by the ability of prekallikrein activation. Prekallikrein (50 μg) was incubated with 1.5 ml of the effluent from gel filtration for 30 min at 37° and kallikrein generated was assayed by BAEe esterase activity.

Assay of kinin. Kinin liberated from samples was assayed on isolated guinea pig ileum suspended in a muscle bath containing 10 ml of oxygenated Tyrode solution using bradykinin as a standard [18]. The ileum was treated with α-chymotryspin, as described by Edery [25], prior to bioassay.

Gel filtration. A 1.5 × 140 cm column of Sephacryl S-300 or a 2.3 × 120 cm column of Sephadex G-100 was equilibrated with 0.02 M Tris buffer (pH 7.4) containing 0.15 M NaCl. The column was eluted with the equilibrating buffer after application of sample. The flow rate was 12 ml/hr and 3 ml fractions were collected. The u.v.-absorbance at 280 nm, BAEe hydrolysis and ability of prekallikrein activation of the effluent fractions were measured.

Immunodiffusion. Immunodiffusion analysis of HMW kininogen was carried out on Ouchterlony agarose plates. Anti-human HMW kininogen serum was kindly supplied by Dr. Nagasawa, Faculty of Pharmaceutical Science, Hokkaido University, Japan.

Chemicals. BAEe and bradykinin were obtained from the Protein Research Foundation, Osaka, Japan; human plasmin and PPAN from A B KABI, Sweden; soy bean trypsin inhibitor (SBTI) and LBTI from Sigma Chemical Co., St. Louis, MO U.S.A.; Trasylol from Bayer, Germany; Trans-aminoethyl-cyclohexene-carboxylic acid (t-AMCHA) from Daiichi Seiyaku, Co., Tokyo, Japan; hexadimethrine bromide (polybrene) from Aldrich Chemical Co., Milwaukee, WI, U.S.A.; Pevikon C-870 from Stockholms Superfosfat Fabriks A. B., Sweden; and agarose from Behring Institut, Germany.

RESULTS

Binding of kallikrein and prekallikrein in plasma to kaolin surface during contact activation. Normal human plasma was incubated with kaolin for varying time periods to study binding of kallikrein and prekallikrein in plasma to the kaolin surface. A maximum binding of the kallikrein was obtained at the end of a 2 min incubation period at room temperature; the surface-bound kallikrein markedly diminished at the end of a 5 min incubation. Whereas in normal plasma 154 mU of the kallikrein was bound to kaolin after a 2 min incubation at room temperature, in HF-deficient plasma the surface-bound kallikrein was undetected. Furthermore, in normal plasma preincubated with polybrene, a known inhibitor of activated HF, the surface-bound kallikrein was markedly diminished with a dose response as shown in Table 1. The data indicate that the binding of kallikrein in plasma to the surface required HF or activated HF. In normal plasma, surface-bound prekallikrein was undetectable after incubation with kaolin, while in HF-deficient plasma or normal plasma preincubated with polybrene, a trace of prekallikrein was bound to the kaolin. These findings suggest that the surface-bound kallikrein derived from activation of the surface-bound prekallikrein by activated HF.

Liberation of kinin from kaolin pellet treated with normal plasma. The washed kaolin pellet was resuspended in the buffer to the original volume and used as a source of kininogen, because the plasma-treated kaolin pellet adsorbed kininogen on the surface, as will be shown in the results of the immunodiffusion study. Treatment of the kaolin pellet with trypsin, plasmin or plasma kallikrein resulted in liberation of kinin as shown in Table 2. No difference in kinin liberation between plasma kallikrein and trypsin indicates that the surface-bound kininogen is HMW kininogen. HMW kininogen has an ability to bind to surfaces, and Jacobsen [26] and Habal *et al.* [27] have reported that low mol. wt kininogen has a low susceptibility to hydrolysis by plasma kallikrein and trypsin. The kaolin suspension alone was incubated for an appropriate time without adding any proteolytic enzymes. A small amount of kinin was always liberated from the kaolin pellet. However, no kinin was liberated in the kaolin suspension preincubated with a kallikrein inhibitor such as SBTI, DFP and Trasylol, suggesting that the kinin liberation is

Table 1. Binding of kallikrein and prekallikrein in plasma to kaolin surface during contact activation*

	Kallikrein	Prekallikrein
Normal plasma without treatment	64	1620
Normal plasma treated with kaolin		
Kaolin pellet	154	0
Supernatant fraction	280	1340
HF deficient plasma without treatment	0	1620
HF deficient plasma treated with kaolin		
Kaolin pellet	0	27
Supernatant fraction	0	1530
Normal plasma treated with polybrene		
Kaolin pellet (1 μg polybrene)	28	<5
Kaolin pellet (10 μg polybrene)	6	<5

* Values are expressed in milliunits of PPAN cleavage. For treatment of normal plasma with polybrene, aliquots of polybrene were added to normal plasma and used immediately for the experiment of incubation with kaolin. Activation of prekallikrein on kaolin surface was performed by adding 10 μg trypsin to the suspension, incubating for 30 min at 37°, and blocking the trypsin with 20 μg lima bean trypsin inhibitor.

Table 2. Liberation of kinin from kaolin pellet treated with normal plasma by proteolytic enzymes*

	Kaolin pellet alone	Kaolin pellet + Trypsin	Kaolin pellet + Plasmin	Kaolin pellet + Kallikrein
Kinin liberation (ng)	9	18	18	17

* Trypsin (10 μg), plasmin (0.05 CU) or plasma kallikrein (5 μg) was incubated with 1.0 ml of the suspension of the kaolin pellet treated with normal plasma for 15 min at 37° and blocked by adding an excess amount of LBTI, t-AMCHA or SBTI, respectively. SBTI was also added to the solution after incubation of the kaolin pellet suspension alone as before.

Table 3. Dissociation of kallikrein from pellet with normal plasma*

	Kaolin pellet alone	Kaolin pellet + Trypsin	Kaolin pellet + Plasmin	Kaolin pellet + Kallikrein
Kaolin pellet	70	28	43	48
Supernatant fraction	59	108	95	83

* Values are expressed in milliunits of PPAN cleavage. Trypsin (10 μg) plasmin (0.05 CU) or plasma kallikrein (5 μg) was incubated with 1.0 ml of the suspension of the kaolin treated with normal plasma for 15 min at 37°. After incubation the samples were separated into kaolin pellet and supernatant fraction. The kaolin pellet was resuspended to the original volume. Trypsin (10 μg/ml) and plasmin (0.05 CU/ml) did not cleave PPAN. The value for the supernatant fraction obtained after treatment with plasma kallikrein was calculated by subtracting the value of plasma kallikrein added from the value of the sample.

attributed to the kininogenase activity of the surface-bound kallikrein.

Dissociation of kallikrein from kaolin pellet treated with normal plasma. The kaolin pellet resuspended to the original volume was incubated with trypsin, plasmin or plasma kallikrein. After incubation, the supernatant fraction was obtained and used for the experiment. Most of the surface-bound kallikrein was dissociated from the kaolin surface by the incubation with proteolytic enzymes; the kallikrein remaining bound to the kaolin surface was less than 50 per cent in each experiment, as shown in Table 3. The kaolin suspension alone was incubated for an appropriate time as control. The surface-bound kallikrein was markedly dissociated, to a lesser degree than after treatment with trypsin, plasmin or plasma kallikrein. The results in Tables 2 and 3 demonstrate that there is a close relationship between kinin liberation and kallikrein dissociation.

Relationship between kinin liberation and kallikrein dissociation from the kaolin pellet. To investigate an interaction with kallikrein and HMW kininogen on the kaolin surface, the kaolin pellet treated with normal plasma alone was incubated without adding any proteolytic enzymes. The kaolin pellet was incubated for varying periods of time. At the end of the desired incubation time, the kaolin pellet and the buffer were separated by centrifugation. After washing, the kaolin pellet was used for determination of the kallikrein remaining bound to the surface. The buffer was used for determination of kinin liberated. The dissociation of the surface-bound kallikrein revealed two phases at a 15 min incubation: the first phase of dissociation which rapidly progressed until the kinin liberation reached a plateau was followed by the second phase where the kallikrein was slowly dissociated and kinin no longer liberated. Thus, the dissociation of the surface-bound kallikrein is closely related to the presence of intact HMW kininogen on the surface. The kinin in the buffer may be liberated not only by the kininogenase activity of the surface-bound kallikrein but also by the kininogenase activity of the kallikrein dissociated from the surface during incubation.

Gel filtration of surface-bound substances on Sephacryl S-300 and Sephadex G-100. The kaolin pellet prepared in a similar manner was allowed to incubate for 60 min to allow dissociation of the surface-bound kallikrein and activated HF. After incubation, the supernatant was applied to a column of Sephacryl S-300. Gel filtration revealed three BAEe esterase peaks and two distinct peaks having the ability of prekallikrein activation. An enzyme demonstrating BAEe esterase activity also possessed the activity of kininogenase and PPAN cleavage. These findings indicate that the enzyme representing BAEe esterase in Fig. 2 is kallikrein. Gel filtration of purified plasma kallikrein with mol. wt of 100,000 on a Sephacryl S-300 column revealed in tubes 55–64 reaching a maximum in tube 58. This result indicates that the third BAEe esterase peak which was found at a mol. wt of 100,000 is free kallikrein. Kallikrein–HMW kininogen complex which will be described below was found at the same tube number as that of the second BAEe esterase peak on Sephacryl S-300 gel filtration, suggesting that the second BAEe esterase peak represents kallikrein–HMW kininogen complex. The first BAEe esterase peak always coincided with the first peak having the prekallikrein-activating ability. As will be described below, the first BAEe esterase peak represents a complex of kallikrein, HMW kininogen and activated HF. The second peak having the prekallikrein activating-ability in Fig. 2 was pooled, concentrated, and applied to a Sephadex G-100 column. Gel filtration revealed two peaks having the prekallikrein-activating ability; their mol. wts were estimated to be approximately 28,000 and 40,000. A small free kallikrein peak was also found at a mol. wt of 100,000, as shown in the inset B of Fig. 2. In the studies of the peak having the prekallikrein-activating ability, the second peak and the first peak were in a ratio of 1:0.32 after a 15 min incubation and in a ratio of 1:1.09 after a 60 min incubation. These findings indicate that free activated HF is largely dissociated from the surface at the first phase described in Fig. 1. The first BAEe esterase peak stored at −20° for 7 days was concentrated and applied to a Sephadex G-100 column. As shown in the inset A of Fig. 2, the second BAEe esterase peak representing free kallikrein was about 27 per cent of total BAEe esterase eluted, while most of the prekallikrein-activating ability was eluted as free forms. This finding indicates that the kallikrein has more stable binding than the activated HF in the kallikrein, HMW kininogen and activated HF complex.

Immunodiffusion of surface-bound substances and components of surface-bound substances. The sus-

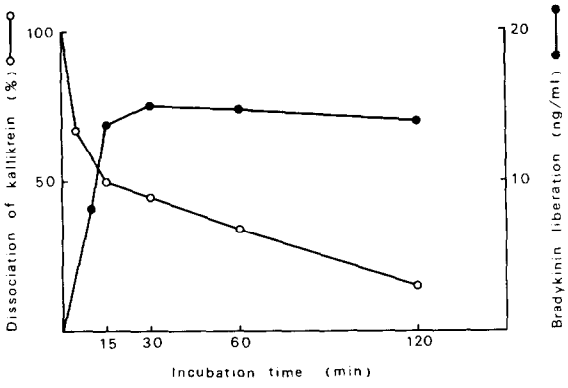

Fig. 1. Dissociation of surface-bound kallikrein and kinin from kaolin pellet treated with normal plasma. The suspension of the kaolin treated with normal plasma was allowed to incubate with shaking for varying times before centrifugation. The kaolin pellet obtained after incubation was resuspended to the original volume. The activity of PPAN cleavage of the kaolin pellet was expressed as per cent; its activity before incubation was defined as 100. The reaction was stopped by adding an excess amount of SBTI at an appropriate time of incubation and kinin liberated in the suspension was measured.

pension of the kaolin pellet treated with normal plasma was incubated with trypsin, plasmin or plasma kallikrein. The kaolin suspension alone was also incubated without adding any proteolytic enzyme. After incubation the supernatant was used for the experiment. In immunodiffusion of HMW kininogen, all of these four samples formed precipitation lines with HMW kininogen antibody, as shown in Fig. 3A. These data indicate that dissociation of the surface-bound HMW kininogen occurs by treatment with trypsin, plasmin or plasma kallikrein. Each of three kallikrein peaks in Fig. 2 was also used for the experiments of immunodiffusion and kinin liberation. In the immunodiffusion study, all of these three samples formed precipitation lines with human HMW kininogen antibody, as shown in Fig. 3B. Treatment of these samples with trypsin did not liberate kinin, indicating the presence of kinin-free HMW kininogen. Thus, the first kallikrein peak was found to contain kallikrein, kinin-free HMW kininogen and activated HF. The fact that a mol. wt of the first kallikrein peak was greater than that of kallikrein–HMW kininogen complex, as shown in Fig. 2, indicates that the first kallikrein peak is a complex of kallikrein, kinin-free HMW kininogen

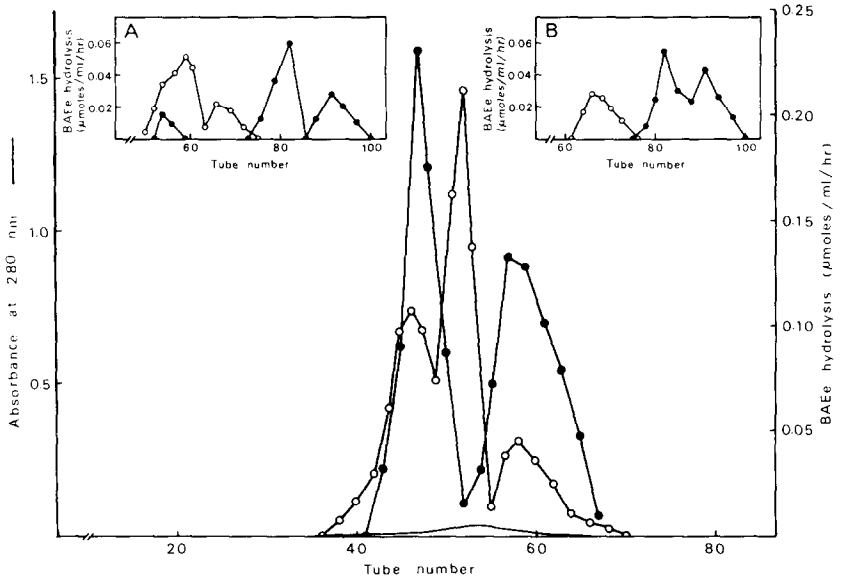

Fig. 2. Gel filtration of surface-bound substances released from kaolin pellet treated with normal plasma. Twenty millilitres of normal plasma was incubated with a kaolin pellet (100 mg) for 2 min at room temperature. The mixture was centrifuged and the supernatant removed. The kaolin pellet was washed two times and resuspended to 10 ml with 0.02 M Tris buffer, pH 7.4, containing 0.15 M NaCl. The kaolin suspension was incubated for 60 min at 37° and the supernatant was applied to a column of Sephacryl S-300. BAEe esterase activity in the effluent was measured for kallikrein. Prekallikrein was incubated with the effluent and BAEe esterase activity in the mixture was measured for prekallikrein-activating ability. Prekallikrein-activating ability was calculated by subtracting the value of kallikrein from the value of the mixture of prekallikrein and the effluent, and was expressed as BAEe hydrolysis. The first BAEe esterase peak containing the prekallikrein-activating ability stored at −20° for 7 days or the second peak having the prekallikrein-activating ability was concentrated and applied to a column of Sephadex G-100. The pattern is shown in the inset A or B. ○——○: kallikrein, ●——●: prekallikrein-activating ability.

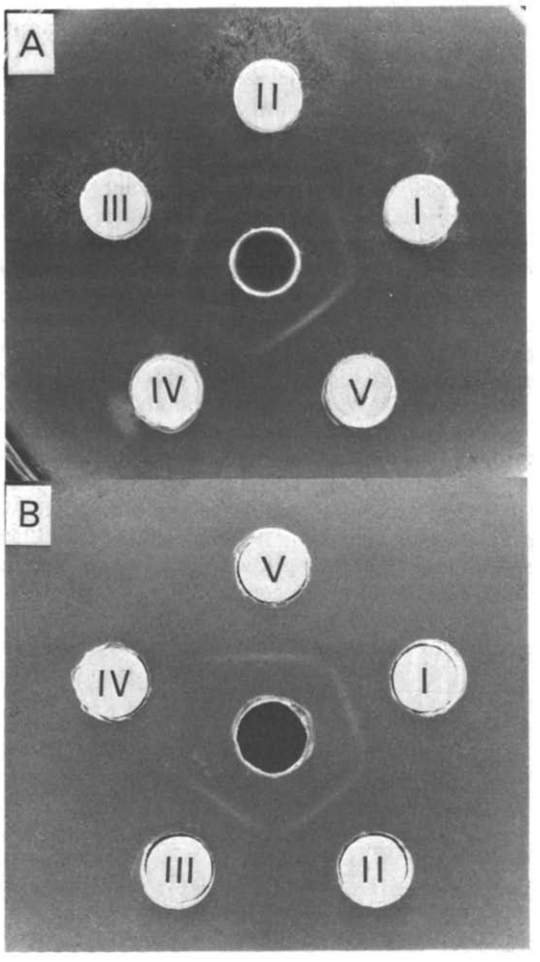

Fig. 3. Double immunodiffusion analysis of the surface-bound substances. Panel A: Trypsin (10 μg), plasmin (0.05 CU) or plasma kallikrein (5 μg) was incubated with 5.0 ml of the suspension of the kaolin pellet treated with normal plasma for 15 min at 37°. The kaolin suspension alone was incubated for 30 min at 37° without adding any proteolytic enzyme. After incubation the samples were separated into kaolin pellet and supernatant. The supernatant was concentrated to about 0.5 ml. I: the surface-bound substances dissociated by treatment with trypsin, II: the surface-bound substances dissociated by treatment with plasmin, III: the surface-bound substances dissociated by treatment with plasma kallikrein, IV: the surface-bound substances dissociated by incubation alone, V: HMW kininogen. The center well contains rabbit anti-human HMW kininogen serum. Panel B: Each of three kallikrein peaks in Fig. 2. was pooled and concentrated to about 0.5 ml. I: the first kallikrein peak (tubes 41–48), II: the second kallikrein peak (tubes 49–54), III: the third kallikrein peak (tubes 55–67), IV: plasma kallikrein, V: HMW kininogen. The center well contains rabbit anti-human HMW kininogen serum.

and activated HF. The third kallikrein peak was also found to contain free kallikrein, kinin-free HMW kininogen and activated HF. This finding suggests the single possibility that kinin-free HMW kininogen and activated HF forms a complex in the third kallikrein peak. The peaks having the prekallikrein-activating ability found on Sephadex G-100 gel filtration of the third kallikrein peak in the inset B of Fig. 2 did not form a precipitation line with human HMW kininogen, indicating that activated HF-HMW kininogen complex of HMW kininogen is not present in the peaks. Therefore, it is considered that kinin-free HMW kininogen in the third kallikrein peak was free.

Binding of kallikrein–kinin-free HMW kininogen complex, prekallikrein or kallikrein to kaolin surface. Kallikrein-kinin-free HMW kininogen complex was prepared by the following method. Thirteen milligrams of prekallikrein-HMW kininogen isolated by gel filtration and Pevicon electrophoresis was incubated with 500 μg of trypsin for 30 min at 37°, blocked the trypsin with an excess amount of LBTI and applied to a Sephacryl S-300 column. Gel filtration revealed one kallikrein peak which did not contain any prekallikrein. Its specific activity of BAEe esterase was 0.036 μmoles/min/mg. The kallikrein peak formed a precipitation line with human HMW kininogen and did not generate any kinin by trypsin treatment. One millilitre of the kallikrein–kinin-free HMW kininogen complex containing 200 μg mixed with 5 mg of kaolin in a plastic tube for 5 min at room temperature to allow binding. After incubation the kaolin pellet was washed two times. There were no kallikrein and HMW kininogen on the kaolin surface, indicating that the kallikrein–kinin-free HMW kininogen complex did not bind to the kaolin surface. Prekallikrein (50 μg) or kallikrein (50 μg) was also incubated with kaolin in a plastic tube. Prekallikrein or kallikrein was undetectable on the kaolin surface.

DISCUSSION

HF, prekallikrein and HMW kininogen are able to bind surfaces [16, 28, 29]. During contact activation, cleavage of surface-bound HF generates two forms of active HF named α-HF_a and β-HF_a; the surface-bound α-HF_a very rapidly cleaves surface-bound prekallikrein [17]. The finding that no prekallikrein was found on the kaolin surface after a 2 min incubation may be mainly due to the complete activation of surface-bound prekallikrein by the ability of surface-bound α-HF_a. The findings that HF-deficient plasma and normal plasma treated with polybrene markedly inhibit the binding of prekallikrein to the kaolin surface suggest that the binding abnormality is closely related to HF or the conformational change in HF yielding α-HF_a [17]. Prekallikrein and HMW kininogen normally circulate in plasma as a complex [15]. The prekallikrein–HMW kininogen complex may bind to the kaolin surface together during incubation and become kallikrein–HMW kininogen complex on the surface by the conversion of prekallikrein to kallikrein. Therefore, it is considered that the kallikrein and HMW kininogen found on the kaolin surface in the study form a complex of kallikrein and HMW kininogen. In fact the presence of kallikrein–HMW kininogen complex on the surface was confirmed by the result that two complexes containing kallikrein and HMW kininogen were found in the supernatant on Sephacryl S-300 gel filtration.

Treatment of the HF molecule with kallikrein resulted in cleavage at two primary sites, yielding three regions termed the c (mol. wt 40,000), d (mol. wt 12,000) and e (mol. wt 28,000) regions; the de and e fragments have enzymatic activity [30, 31]. Two activated HFs which possesses a mol. wt of 40,000 and 28,000 as shown in Fig. 2 are probably the de and e fragments of HF, respectively. The presence of the kallikrein–HMW kininogen-activated HF complex in Fig. 2, the requirement of HMW kininogen for binding of prekallikrein to the kaolin surface [16], the enhancement of binding of prekallikrein–HMW kininogen complex by HF in Table 1, the absence of binding ability of prekallikrein or kallikrein to the kaolin surface, and the absence of binding ability of prekallikrein to HF (unpublished data) suggest that the HMW kininogen in the kallikrein–HMW kininogen-activated HF complex directly bind to the de or e region of HF [31] on the kaolin surface. In Fig. 2, both the kallikrein activity and the prekallikrein-activating ability in the kallikrein–HMW kininogen-activated HF complex were present in a ratio of 1: 1.19, and both the kallikrein activity in the second and third kallikrein peaks and the prekallikrein-activating ability in its second peak in a ratio of 1: 1.04. These findings also suggest that most of the surface-bound prekallikrein–HMW kininogen complex binds directly to the active region of HF on the kaolin surface. Thus, the binding of the surface-bound HF to the HMW kininogen in the surface-bound prekallikrein–HMW kininogen may form a ternary complex on the surface. This ternary complex must be favourable for HMW kininogen to enhance the cleavage of HF by kallikrein [11] and the activity of the activated HF [13].

The ternary complex binding to the surface with HMW kininogen and HF may be dissociated from the surface by cleavage of HMW kininogen and HF by kallikrein, yielding a complex of kallikrein, kinin-free HMW kininogen and activated HF in fluid. Because kallikrein–kinin-free HMW kininogen complex and activated HF have no ability to bind to surface. An enhancing effect of HMW kininogen on the activation of surface-bound HF by kallikrein probably requires for the intact molecule of HMW kininogen. Chan *et al.* have reported that the cleavage of HMW kininogen by kallikrein decreased the enhancing effect of the activation of HF [32]. The dissociation of the surface-bound kallikrein revealed two phases: the first phase with rapid dissociation and the second phase with slow dissociation. One can expect the enhancing effect of HMW kininogen on cleavage of the surface-bound HF at the first phase, but not at the second phase where kinin was totally liberated from the surface-bound HMW kininogen. This may be a reason why the dissociation of the surface-bound kallikrein revealed two phases. The free kallikrein in Fig. 2 may derive not only from the ternary complex of kallikrein, HMW kininogen and activated HF but also from the kallikrein–HMW kininogen complex in fluid phase, yielding HMW kininogen–activated HF complex and HMW kininogen. But the HMW kininogen–activated HF complex was not found in the supernatant fraction, as shown in Fig. 2. This finding may be attributed to the weaker binding of HMW kininogen to activated HF. Thus, the dissociation of kallikrein (complex) from the normal plasma-treated kaolin surface may be initiated either locally by the cleavage of the surface-bound HF and HMW kininogen by the surface-bound kallikrein or in the fluid phase by kallikrein (complex) dissociated from the surface. The addition of trypsin, plasmin or plasma kallikrein to the kaolin pellet suspension, as shown in Table 3, probably facilitates an enzymatic cleavage of the surface-bound HF and HMW kininogen in fluid.

Contact activation of normal plasma which involves the dissociation of surface-bound kallikrein may be explained by the following. It requires, as the initial step, the binding of HF to the kaolin surface, yielding an active form of HF (αHF_a) [17]. HMW kininogen binds to the kaolin surface, placing prekallikrein on the kaolin surface, since prekallikrein and HMW kininogen are complexed in plasma [15]. The HMW kininogen also binds to the e region [31] of the surface-bound HF, yielding a ternary complex of HF, prekallikrein and HMW kininogen on the kaolin surface. The HMW kininogen which has two binding sites easily accelerates the activation of prekallikrein by the surface-bound α-HF_a and the activation of HF by kallikrein in the solid and fluid phases. Direct interaction of kallikrein with HMW kininogen and HF on the kaolin surface results in kinin liberation and generation of activated HF, and dissociates a complex of kallikrein, kinin-free HMW kininogen and activated HF in fluid. The dissociation of kallikrein from the kaolin surface rapidly progresses until intact HMW kininogen is depleted, yielding two types of activated HF which correspond to the de and e fragments [31]. The binding of activated HF and kallikrein to HMW kininogen is not stable, the activated HF has much weaker binding than the kallikrein. The dissociation of the ternary complex of kallikrein, kinin-free HMW kininogen and activated HF yields kallikrein–kinin–free HMW kininogen complex, kallikrein, kinin-free HMW kininogen and two activated HFs in fluid.

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