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ACTIVATION OF DOG PLASMA KALLIKREIN WITH GLASS AND ACETONE

MASAO NAKAHARA

Department of Orthopaedic Surgery, Sapporo Medical College, Japan

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Abstract—The activities of glass-activated and acetone-activated plasma kallikreins from dogs were determined by measuring both kininogenase and toluene sulphonyl-arginine methyl ester (TAMe) esterase activities. Glass-activated plasma was centrifuged to sediment the glass beads and the adsorbed components were eluated. Although acetone activation of kininogenase is impossible after previous glass treatment, the addition of the eluate to the original supernatant (100 mg glass beads/ml of plasma) facilitated acetone activation with regard to kininogenase, TAMe esterase, and fibrinolytic activities. Furthermore, when the eluate was added to the original supernatant from plasma with massive surface contact (600 mg glass beads/ml of plasma), kininogenase activity in the supernatant could be accelerated. This finding suggests that the precursor of Vogt's kininogenase I is functionally the same as that of kininogenase II. Glass-activated plasma kallikrein was partially purified by chromatography on DEAE cellulose, DEAE-Sephadex A-50, and CM-Sephadex C-50. The results indicate that glass-activated plasma kallikrein is probably identical with acetone-activated plasma kallikrein; both kallikreins had similar ratios of hydrolytic activity on various synthetic esters such as TAMe, benzoyl-arginine methyl ester, tosyl-lysine methyl ester, and acetyl-lysine methyl ester. Both enzymes were completely inhibited by trasylol and soy bean trypsin inhibitor, not inhibited by lima bean trypsin inhibitor, and inhibited about 60% by naturally occurring plasma inhibitors at a final concentration of 0·2 mg protein/ml. When treated with acid at pH 4 at 37° for 30 min, 60% of their activity was lost. The enzymes did not release kinin from Jacobsen's substrate 2. The ratio of kininogenase activity/TAMe-hydrolysis of the enzymes were constant. The molecular weight of the enzymes was estimated to be approximately 100,000 by gel filtration on a Sephadex G-200 column.

The kinin forming system can be activated by many agents such as glass [1, 2], acetone [3–5], acid [6, 7], casein [8], celeite [9], chloroform [10], antigen–antibody aggregates [11, 12], plasmin [13, 14] and trypsin [15]. Some of the agents can activate not only one kallikrein but also two or more kallikreins including prekallikrein activators. Two different kinin-forming systems have recently been postulated by Vogt on the basis of functional analysis of intrinsic plasma kinin formation; glass-activated plasma kallikrein originates from kininogenase II and acetone-activated plasma kallikrein from kininogen I [16]. Although it seems that many kinds of kallikreins exist independently, there are few studies which compare their properties. Recently, Collins *et al.* have reported that they could not fully exclude the possibility that the kallikrein adsorbed onto glass differs functionally from the kallikrein which is activated with acetone or acid [17]. In this study, glass and acetone have been chosen from many agents which activate the kinin-forming system in plasma and the present experiments are designed to examine the possibility of the identity of glass-activated plasma kallikrein and acetone-activated plasma kallikrein.

MATERIALS AND METHODS

Blood. Mongrel dogs weighing 14–18 kg were used for the experiments. Using a silicone technique, blood samples from the femoral vein were collected in 0·1 vol of 3·1% sodium citrate and centrifuged at 3,000 rev/min for 30 min at 4°. The separated plasma were pooled and stored in polyethylene tubes at 4°.

Activation of plasma. Fresh plasma was shaken with 0·1 mm diameter glass beads at room temperature for 10 min using a mechanical shaker, of not otherwise stated. After centrifugation at 3,000 rev/min for 10 min, the supernatant was removed with siliconized pipettes. The glass beads were washed repeatedly with 0·15 M NaCl until the washings had an optical density at 280 nm of less than 0·05. Subsequently, the substances adsorbed onto the glass beads were eluted by the repeated addition of small quantities of 1·0 M NaCl in 0·1 M tris buffer, pH 8·0, until the optical density of the eluate was less than 0·05. The latter washings were pooled and concentrated to half the volume of the starting plasma, using an Amicon Ultrafiltration Chamber with a UM-10 membrane after dialysis overnight at 4° against 0·9% saline solution.

Acetone activation of fresh plasma or supernatant obtained after glass activation was carried out by adding acetone to plasma (20% v/v of acetone) in a siliconized test tube [18]. The mixture was allowed to stand at room temperature for 17 hr. The acetone was then evaporated in a rotary evaporator. After evaporation the acetone-activated plasma was reconstituted to the original volume with deionized water.

Plasma inhibitors. The supernatant obtained from plasma brought to 50% saturation with ammonium sulphate was subjected to dialysis overnight at 4° against 0·9% saline solution [19].

Kininogen. Kininogen substrate was prepared as follows: heat-inactivated plasma, prepared by heating fresh plasma at 60° for 60 min in order to destroy kallikreins, their activators and prekininogenase [7],

was incubated at 37° for 30 min at pH 2 to destroy kininogenase inhibitors [20] and then neutralized. The resulting precipitate was eliminated by centrifugation. A partially purified preparation of kininogenase (substrate 2) was also prepared by the purification procedure of Jacobsen and Kriz, up to stage IV [21].

Assay of kinins. Kinins released by the kininogenases were assayed on isolated guinea-pig ileum suspended in a muscle bath containing 10 ml of oxygenated Tyrode solution using synthetic bradykinin as a standard [22]. The ileum was treated with α-chymotrypsin as described by Edery [23] prior to bioassay.

Estimation of kininogenase activity. To 0·2 ml of kininogen substrate in a polyethylene tube were added 0·1–0·4 ml of samples, 0·1 ml of 1,10-phenanthroline (final concn 10^{-4} M) and 0·3–0·6 ml of 0·1 M tris buffer, pH 7·8. After incubation at 37° for 10 min the reaction was stopped by the method of Briseid *et al.* [24]. The activity is expressed as ng bradykinin/ml plasma or sample solution. Liberated kinins were found to be inactivated by incubation with α-chymotrypsin.

Measurement of esterase activity. Esterase activity was measured by determining the methanol released from the substrate, tosyl-arginine methyl ester (TAMe), benzol-arginine methyl ester (BAMe), tosyl-lysine methyl ester (TLMe), or acetyl-lysine methyl ester (ALMe) (final concn 0·015 M) [25, 26]. Esterase activity is expressed in μmoles of ester hydrolysed per hr per ml test solution.

Euglobulin clot lysis. Fresh plasma was diluted 20-fold with deionized water and the pH adjusted to 5·3 with 1% acetic acid. After centrifugation the sediment was dissolved in a volume of 0·1 M phosphate buffer, pH 7·8, equal to half the volume of the original plasma and used as the euglobulin fraction. Plasminogen-free fibrinogen was prepared by lysine–sepharose chromatography [27] of bovine fibrinogen. The lysis time of euglobulin clots at 37° was recorded; 0·2 ml of euglobulin solution was mixed with 0·5 ml of 0·5% bovine fibrinogen and 0·1 ml of bovine thrombin (50 NIHU/ml).

Protein. The protein content of the solutions was determined by applying the formula of Kalcker [28] to the optical density at 260 and 280 nm.

Column chromatography. The plasma activated with glass or acetone was diluted 2-fold with deionized water and dialyzed overnight at 4° against 0·05 M NaCl. The dialyzed plasma was subjected to column chromatography on a 3·5 × 47 cm polyethylene column (LKB) of diethylaminoethyl (DEAE) cellulose which was equilibrated with 0·05 M NaCl containing 0·075 M phosphate buffer, pH 8·0. The column was eluted with a linear gradient of increasing concentrations of NaCl (0·05–0·7 M). To produce the NaCl gradient, two connected containers were used; 400 ml of 0·05 M NaCl in 0·0075 M phosphate buffer, pH 8·0, was placed in the first container and an equal volume of 0·7 M NaCl in 0·0125 M phosphate buffer, pH 8·0, in the second container of the mixing chamber. The flow rate was 20 ml/hr and 5 ml fractions were collected. The u.v.-absorption at 280 nm, TAMe esterase and kininogenase activities of the effluent fractions were measured. Fractions in the first protein peak which revealed TAMe esterase and kininogenase activities were pooled and concentrated to about 15 ml using an Amicon Ultrafiltration Chamber with a UM-10 membrane. They were chromatographed then on a 3·5 × 45 cm polyethylene column (LKB) of DEAE–Sephadex A-50 which was equilibrated with 0·1 M NaCl containing 0·05 M phosphate buffer, pH 8·0. The column was eluted with a linear gradient of increasing concentrations of NaCl (0·1–0·7 M). To produce the NaCl gradient, three containers connected in series were used; 390 ml of 0·1 M NaCl in 0·05 M phosphate buffer, pH 8·0, was placed in the first container, and equal volumes of 0·4 and 0·7 M NaCl in 0·05 M phosphate buffer, pH 8·0, were placed in the second and the third containers of the mixing chamber, respectively. The flow rate was 15 ml/hr and 3 ml fractions were collected. The effluent was assayed as before. Fractions which revealed TAMe esterase and kininogenase activities in the third protein peak were pooled and concentrated to about 8 ml by ultrafiltration as before. The solution was chromatographed next on a 1·5 × 50 cm column of CM–Sephadex C-50 which was equilibrated with 0·015 M phosphate buffer, pH 6·5. To produce the NaCl gradient, two connected containers were used; 100 ml of 0·015 M phosphate buffer, pH 6·5, and 100 ml of 0·2 M NaCl containing 0·015 M phosphate buffer, pH 6·5, were placed in the two containers of the mixing chamber. The flow rate was 10 ml/hr and 3 ml fractions were collected. Fractions which revealed TAMe esterase and kininogenase activities were pooled and used as partially purified kallikrein.

Chemicals. TAMe and TLMe were obtained from the Protein Research Foundation, Osaka, Japan; BAMe, ALMe, soy bean trypsin inhibitor (SBTI) (1 mg inhibits about 1 mg trypsin), bovine thrombin, lima bean trypsin inhibitor (LBTI) and glass beads from Sigma Chemical Co., U.S.A.; Trasylol® and Kallikrein® from Bayer, Germany; bovine fibrinogen from Armour, U.S.A.; bovine albumin and human gamma globulin from Miles Lab. Inc., U.S.A.; α-chymotrypsin from Eisai Co., Japan. Bradykinin was kindly supplied by Sandoz, A. G., Basel, Switzerland.

RESULTS

Glass and acetone activation of plasma. Glass activation of dog plasma is limited by the presence of potent kininogenase inhibitors in the plasma. The supernatant of glass-activated plasma released kinin ranging from 5 to 20 ng/10 min/0·1 ml. Plasma obtained from one dog was used in a series of experiments.

Acetone activation of kininogenase is impossible after previous glass treatment in spite of the fact that kininogenase can be activated by glass activation after previous acetone treatment [29]. This was confirmed as shown in Table 1 (Expt. 3 and 6). When acetone activation was performed after adding the eluate from glass beads to the supernatant (100 mg glass beads/ml of plasma) kininogenase activity could be accelerated markedly (Expt. 4). TAMe esterase activity was elevated also following kininogenase activation, although there was no quantitative parallel between kininogenase activity and TAMe esterase activity.

Table 1. Kininogenase activity, TAMe esterase activity and euglobulin clot lysis time in glass-activated and acetone-activated plasma

Samples used (ml)	Kininogenase activity (ng/10 min)*	TAMe esterase activity (μM/hr)*	Euglobulin clot lysis time (min)†
1. Supernatant from glass-activated plasma (0·1)	16	0·59	225–240
2. Eluate from glass beads (0·05)	5	0·17	
3. (1) – acetone activation (0·1)	76	1·44	22
4. (1) + (2) – acetone activation (0·15)	20	0·81	40
5. Acetone-activated plasma (0·1)	48	0·72	20
6. (5) – glass activation (0·1)	65	1·20	21
7. Plasma (0·1)	< 10	0·38	480–600

* An amount corresponding to 0·1 ml plasma was used for the assay of kininogenase and TAMe esterase activities.
† 0·2 ml euglobulin (see Materials and Methods) was used for this study.

These findings indicate that the phenomenon is due to an adsorption of important components for acetone activation onto glass surfaces. The eluate also induced acceleration of fibrinolytic activity during acetone activation (Expt. 3 and 4) as well as acetone alone (Expt. 5). Effects of acetone on fibrinolysis may be due partly to the destruction of plasmin inhibitors. Webster and Pierce reported that acetone failed to activate plasminogen as measured by its proteolytic activity on azocasein [13]. The discrepancy between these results may be attributed to the different plasmin substrates.

Kininogenase and TAMe esterase activities in the supernatant and the eluate prepared from plasma with massive surface contact. The ineffectiveness of repeated glass contact of plasma on kininogenase has been explained by consumption of a specific kininogen during glass contact and by prekallikrein. The findings in Table 1 contradict this. Therefore plasma were prepared with massive contact of glass beads (600 mg/ml of plasma); the supernatant, free of kininogenase II and kininogen II, must contain prekininogenase I which is activated with acetone according to Siedel *et al.* [29].

The adsorbed substances were eluated using glass beads obtained after the first glass treatment. In order to eliminate kininogenase inhibitors, the supernatant was treated with acid [20] or acetone [24]. As shown in Table 2, the treated supernatants were mixed with various amounts of the eluate; in experiments 2 and 6, the proportion of supernatant to eluate was equal to untreated plasma. With an increasing proportion of eluate kininogenase activity was accelerated without any further acetone treatment. TAMe esterase activity also was elevated by adding portions of eluate. These data strongly suggest that the limitation is due to components adsorbed onto glass surfaces indicating that the eluate is responsible for glass activation. Jahrreiss and Habermann have reported that a limiting factor in glass activation is prekallikrein since kinin-formation is increased by adding purified prekallikrein to glass-activated plasma treated previously with acid [30].

Purification of glass-activated and acetone-activated plasma kallikreins

Chromatography on DEAE cellulose. The same plasma was always used for glass and acetone activations. Typical eluation patterns from columns of

Table 2. Kininogenase and TAMe esterase activities in the supernatant and the eluate from plasma with massive contact

Sample used (ml)	Kininogenase activity (ng/10 min)	Acceleration (%)§	TAMe esterase activity (μM/hr)	Acceleration (%)§
1. Acid-treated supernatant† (0·10)*	24	—	0·17	—
2. (1) + eluate (0·05)	33	37	0·30	41
3. (1) + eluate (0·10)	42	75	0·46	100
4. (1) + eluate (0·20)	55	129	0·74	194
5. Acetone-treated supernatant‡ (0·10)*	17	—	0·88	—
6. (5) + eluate (0·05)	22	29	12·0	30
7. (5) + eluate (0·10)	26	53	1·44	50
8. (5) + eluate (0·20)	34	100	1·62	57
9. Eluate (0·05)	Negligible		0·06	

* An amount corresponding to 0·1 ml of the original volume of the supernatant was used for the experiments. Samples 1–9 were preincubated at 37° for 20 min prior to the assay.
† Supernatant, adjusted to pH 2·0, was incubated at 37° for 30 min and then neutralized.
‡ Supernatant was treated with acetone as well as plasma (see Materials and Methods).
§ Values of eluates were substracted from the values of 2, 3, 4, 6, 7, 8 and 9, respectively, and the per cent acceleration calculated.

DEAE cellulose of gass-activated and acetone-activated plasma are shown in Fig. 1, a and b, respectively. Two protein peaks were found; each protein peak had kininogenase and TAMe esterase activities. The first protein peak constantly contained about 70% of the total kininogenase activity recovered in the two protein peaks, although kininogenase recovered from different plasma varied in potency. When the supernatant was treated with 50% ammonium sulphate to eliminate kallikrein inhibitors [31], the second protein peak almost disappeared in both glass-activated and acetone-activated plasma. By using plasma [32] or acid-treated plasma as substrate, it was confirmed that the second peak contained prekallikrein activator. Fractions in the first protein peak showing kininogenase and TAMe esterase activities were selected and pooled for further purification of plasma kallikrein.

Chromatography on DEAE–Sephadex A-50. The pooled fractions were concentrated to 15 ml by ultrafiltration and applied to a DEAE–Sephadex A-50 column. Representative patterns of absorbance at 280 nm of the eluates are shown in Fig. 2, a and b. Most of the kininogenase and TAMe esterase activity was recovered in the third protein peak. Fractions containing kininogenase activity also revealed TAMe esterase activity as shown in Fig. 2a. The distribution of enzyme activity in relation to the eluated proteins was similar in all experiments, but minor peaks occasionally appeared in tubes 100–170. The second TAMe esterase peak in Fig. 2b contained low kininogenase activity and prekallikrein activator, as did the second protein peak in the DEAE cellulose chromatogram.

Chromatography on CM–Sephadex C-50. Each fraction in the third protein peak above containing kininogenase and TAMe esterase was dialyzed against 0·015 M phosphate buffer, pH 6·5, at 4° overnight and concentrated by ultrafiltration. The solution was applied to a CM–Sephadex C-50 column after adjusting to pH 6·5. The distribution of protein and enzyme activity in the eluate is shown in Fig. 3, a and b. Glass-activated or acetone-activated plasma kallikrein was eluted in the descending limb of the second protein peak as a single peak. These kallikreins were pooled and adjusted to pH 8·0 for further studies. Kininogenase activity in glass-activated and acetone-activated plasma was purified 79-fold and 107-fold, respectively, as shown in Table 3. No plasminogen, plasmin and thrombin could be detected in these partially purified preparations. The hydrolysis of four esters at a single substrate concentration, under similar test conditions, was tested using the two kinds of partially purified kallikreins. As shown in Table 4, no substantial difference was observed between glass-activated plasma kallikrein and acetone-activated plasma kallikrein.

Kininogenase activity cannot be compared with TAMe-hydrolysis on the basis of the activity per mg of preparations because partially purified kallikreins are contaminated with gamma-globulin [33]. Therefore, an appropriate amount of effluent which produced 100 ng bradykinin/10 min, using the highest fraction (No. 28 in Fig, 3, a and b), was used for TAMe-hydrolysis; 0·42 ml and 0·14 ml of effluent were used for glass-activated plasma kallikrein and acetone-activated plasma kallikrein, respectively. TAMe-hydrolysis of these two samples which varied in volume had no difference in potency, giving identical kininogenase activity/TAMe-hydrolysis ratios as shown in Table 5.

The kininogenase activities off glass-activated and acetone-activated plasma kallikreins were inhibited 58% and 61% respectively by a naturally occurring plasma inhibitor at a final concentration of 0·2 mg protein/ml. They were completely inhibited by trasylol and SBTI at a final concentration of 10 μg/ml, but were not inhibited by LBTI up to a final concentration of 100 μg/ml. They lost 64% and 68% of their activity, respectively, after acid treatment at pH 4 and 37° for 30 min.

According to Jacobsen, substrate 2 is a kininogen which releases kinin rapidly only with glandular kallikrein and slowly with plasma kallikrein [34]. When kininogen substrate was replaced by substrate 2, neither glass-activated nor acetone-activated plasma kallikrein generated any kinin during a 10-min incubation. The discrepancy between Jacobsen's substrate 2 and other kininogens, which were introduced by many authors as described in Habermann's survey [35], is not the case in this experiment.

The molecular weight of partially purified glass-activated and acetone-activated kallikreins was determined by gel filtration on a Sephadex G-200 column. SBTI, bovine albumin, and human gamma-globulin were used as protein markers. The molecular weight of a peak having kininogenase and TAMe esterase

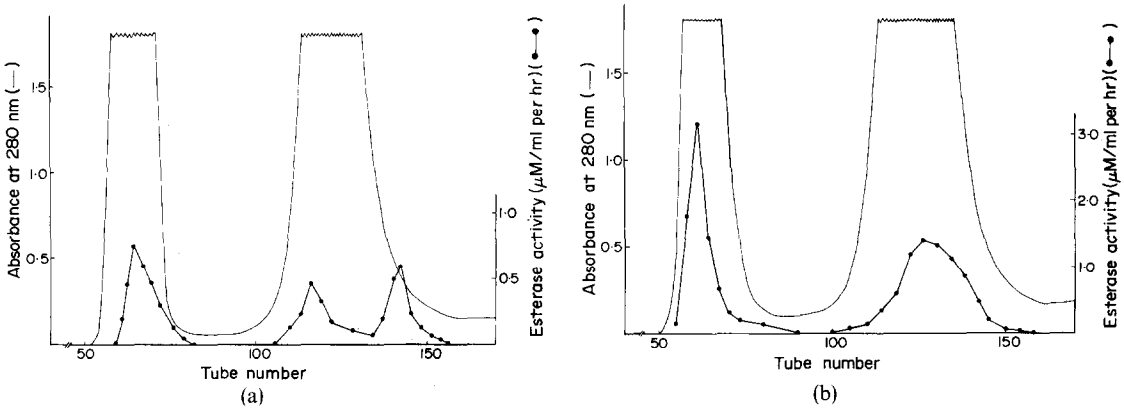

Fig. 1.

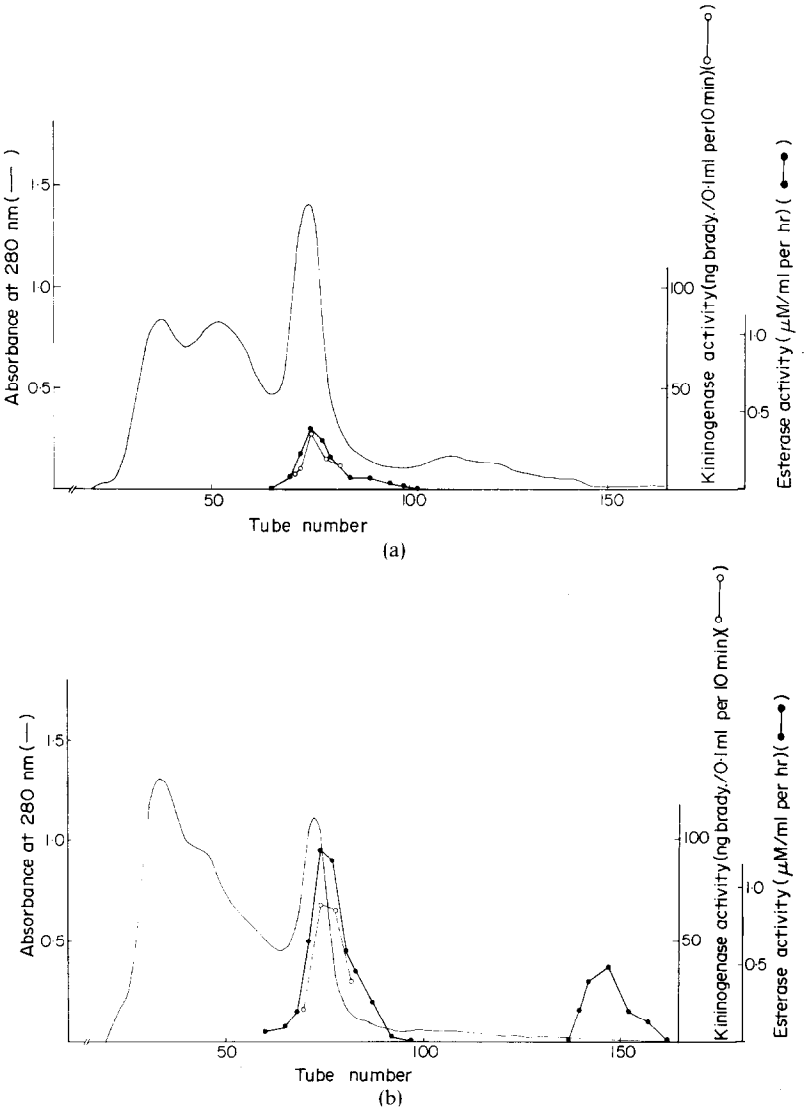

Fig. 2.

activities was estimated to be about 100,000 in agreement with various other plasma kallikreins described in previous reports [36–38].

DISCUSSION

It is generally accepted that kininogenase activation with glass or acetone starts with activation of the Hageman factor (HF) [2, 5], as kininogenase activity cannot be generated in HF deficient plasma [39]. The plasma of dogs contains several other species of HF [31, 40].

In recent years it has been suggested that certain components which participate in the kinin-forming system, such as HF [5], activated HF [5], prekallikrein activator [9], HF-cofactor [41] and kallikrein [17, 42] are adsorbed onto surfaces; HF in plasma is activated by contact exposure [5]. Activated HF and HF-cofactor generate fibrinolytic activity by the conversion of plasminogen to plasmin [41], which in

Table 3. Purification of glass-activated and acetone-activated kallikreins by column chromatography

	Protein (mg)		Total kininogenase activity (ng)		Specific activity (ng/mg protein)		Degree of purification	
	Glass	Acetone	Glass	Acetone	Glass	Acetone	Glass	Acetone
Plasma (17 ml)	680	680	2720	8160	4	12	1	1
DEAE cellulose	139	160	12700	50300	91	320	25	26
DEAE–Sephadex A-50	50	40	8900	34400	178	860	45	72
CM–Sephadex C-50	9	11	2835	14160	315	1280	79	107

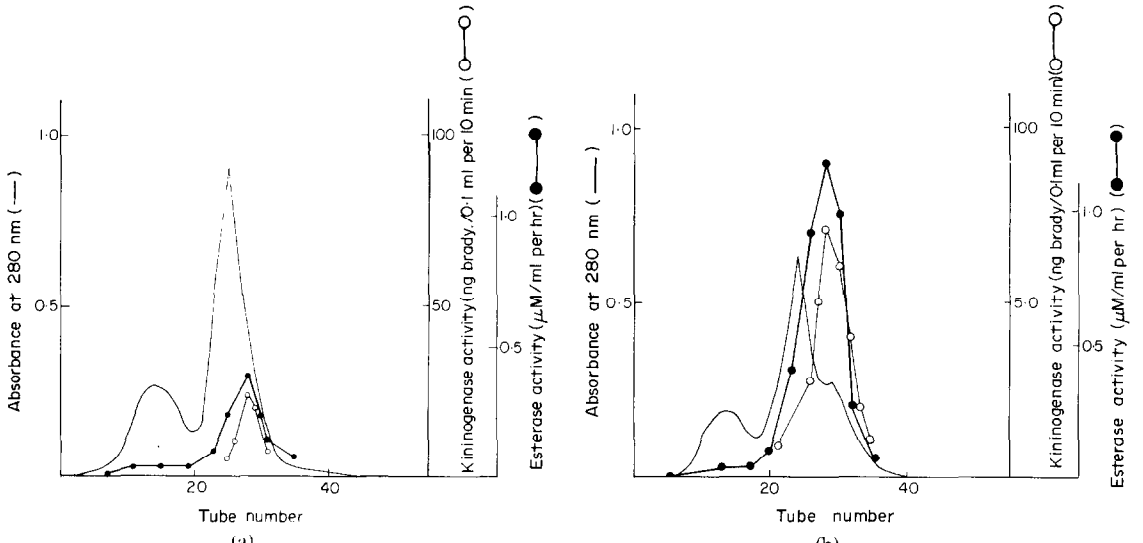

Fig. 3.

turn generates prekallikrein activator by the digestion of activated HF [43]. Prekallikrein activator is capable of converting plasma prekallikrein to plasma kallikrein [44–46] and plasminogen proactivator into its active state [39]. Therefore, it is considered that the eluates from glass beads must contain these components. As shown in Table 1, addition of the eluate to the original supernatant makes acetone activation possible in regard to kininogenase, TAMe esterase and fibrinolytic activities. The lack of acetone activation after previous glass treatment can be explained from this result. However, this result still supports the possibility of two kinin-forming systems in plasma, considering that kininogenase I remains intact during glass contact and can be activated by acetone. For this reason glass activation of plasma was carried out with massive contact of glass beads.

If the supernatant prepared from plasma after massive surface contact is activated with the eluate alone, the theory of two kinin-forming systems [16] is weakened. As shown in Table 2, a kininogenase in the supernatant can be activated with the eluate alone. The kininogenase activated in this manner should be kininogenase II, as if prekininogenase II were contained in the supernatant. But the supernatant in Table 2 should not contain prekininogenase II due to the massive contact with glass beads as described by Siedel *et al.* [29]. These findings indicate that kininogenase I and kininogenase II may be the same, suggesting that some of the precursor of kininogenase still remained during massive surface contact. This consideration obviously disagrees with two kinin-forming systems postulated by Vogt and his colleagues [16, 47, 48]. Prekallikrein activator has been

Table 4. Hydrolysis of various esters by partially purified kallikreins in glass-activated and acetone-activated plasma

	Substrates			
	TAMe	BAMe	TLMe	ALMe
Glass-activated plasma kallikrein*	100	85	83	89
Acetone-activated plasma kallikrein*	100	85	81	82

Values are expressed in per cent of TAMe esterase activity.
* Fractions showing higher kininogenase and TAMe esterase activities in Figs. 3a and b were pooled, respectively, and used for the experiment.

Table 5. Ratio of kininogenase activity/TAMe-hydrolysis of partially purified kallikreins

	Effluent from CM-Sephadex C-50,* volume used (ml)	Kininogenase activity (ng/10 min)	TAMe esterase activity (μM/hr)	Kininogenase activity / TAMe-hydrolysis
Glass-activated plasma kallikrein	0·42	100	0·26	0·38
Acetone-activated plasma kallikrein	0·14	100	0·26	0·38

* Fraction 28 in Figs. 3a and b was used, respectively.

isolated from human plasma after surface contact [9] or acetone activation [39]. Most of the activated HF is converted to the low molecular weight prekallikrein activator by massive contact exposure [9].

Several investigators have reported two or more kininogenases coexisting in the same plasma. Webster has fractionated, by DEAE cellulose chromatography, five enzymes able to generate kinin from fresh plasma. The initial protein peak, having little or no binding affinity for cellulose, contained enzyme I which was identified as plasma kallikrein [49]. Colman *et al.* isolated chromatographically three kaolin-activated kallikreins [36], but the procedures of identification raise a strong suspicion whether the identified enzymes are kallikrein, prekallikrein activator, or non-specific esterase as described by Movat *et al.* [46] and Jahrreiss and Habermann [30]. Furthermore, Movat *et al.* demonstrated that three permeability-enhancing and kinin-forming regions were fractionated by electrophoresis after incubating guinea-pig serum with antigen–antibody aggregates [11, 12]. Lately, Movat *et al.* have isolated six enzymes from human plasma including kallikrein, complexed kallikrein and an activator of prekallikrein [46]. Eisen and Glanville described two kallikreins using chromatography on DEAE cellulose; kallikrein and kallikrein + prekallikrein activator [32]. Yano *et al.* also isolated chromatographically three fractions and showed that two of them contained prekallikrein activator in casein-activated bovine serum [37]. These studies, using chromatography and electrophoresis, suggest the existence of one native kallikrein in the blood. On the other hand, two kinin-forming systems have been postulated on the basis of functional analyses by Vogt and his colleagues [16, 47, 48]. According to them, glass-activated kininogenase II and acetone-activated kininogenase I have not only their own precursor of kininogenase I and II but also kininogen I and II. There are few investigations in which the relationship between functional kallikrein (Vogt) and isolated kallikreins generated by various modes of activation has been discussed intensively. Henriques *et al.* purified chromatographically horse plasma kallikrein adsorbed onto glass beads and suggested that glass-activated and acid-activated plasma kallikreins were the same substance [42]. Yano *et al.* indicated that the kininogenase activities of casein-activated and glass-activated bovine kallikrein showed the same tendency toward kininogen I and kininogen II [37]. This study provides evidence that glass-activated plasma kallikrein is probably identical with acetone-activated plasma kallikrein on the basis of chromatographic behaviour, similarities in behaviour of both kallikreins towards various synthetic esters, inhibitors, kininogen (substrate 2) and acid treatment, and a constancy of the ratio of kininogenase activity/TAMe-hydrolysis of both kallikreins.

In conclusion the present findings suggest that the generation of plasma kallikrein by glass or acetone activation originate from the same precursor. However, the question as to whether Vogt's two specific kininogen I and II are present or not remains unanswered.

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