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Kinin-inactivating Enzyme from the Mushroom *Tricholoma conglobatum*. IV. Its Effects on the Kallikrein-Kinin System[1)]

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A potent kinin-inactivating enzyme, Shimeji kininase, may have applications in research on the kallikrein–kinin system in the body. In order to test its suitability, the effects of this enzyme, in addition to kinin destruction, on substances related to the kallikrein–kinin system were investigated.

This enzyme hardly affected the esterase activities of the glandular kallikreins.

It destroyed the heat-denatured kininogens of various species, but did not attack the native rat low molecular weight kininogen molecule.

The vasodilative activity of this enzyme assayed in the dog was almost negligible, and no kinin activity could be detected in incubation mixtures of this enzyme and heat-denatured kininogens of various species by the Magnus method. Therefore, this enzyme does not have kininogenase activity, and its destruction of the heat-denatured kininogens was not due to kinin liberation from the kininogens.

Intravenous injection of this enzyme in the rat caused the depletion of plasma high molecular weight kininogen, presumably due to plasma prekallikrein activation, while the low molecular weight kininogen level in plasma was not significantly changed.

Keywords——kininase from mushroom *Tricholoma conglobatum*; glandular kallikrein; plasma kallikrein; high molecular weight kininogen; low molecular weight kininogen; kinin liberation; activation of plasma prekallikrein; plasma kininogen depletion

Previously we reported that a kind of mushroom, *Tricholoma conglobatum* (Shimeji, in Japanese), contained potent kinin-inactivating activity, and this enzyme, named Shimeji kininase, was purified.[3)] This enzyme was stable in the rat and markedly suppressed the action of kinin in the body,[1)] so it may be useful in research on the role of the kallikrein–kinin system in the body. However, before practical application of this enzyme, studies of possible effects on the kallikrein–kinin system in addition to the destruction of kinin should be examined.

In the present paper, the authors describe the effects of this enzyme on substances related to the kallikrein–kinin system.

Materials and Methods

Materials——Synthetic bradykinin and N^{α}-benzoyl-L-arginine ethyl ester (BzArgOEt) were supplied by the Protein Research Foundation (Osaka), and trypsin (2 × crystallized) was obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Mepyramine maleate, atropine sulfate and soybean trypsin inhibitor (SBTI) were products of Poulenc Ltée-Ltd. (Montreal, Canada), Iwaki Seiyaku Co. (Tokyo) and Sigma Chemical Co., respectively. Highly purified hog pancreatic kallikrein (hog PK),[4)] human urinary kallikrein (HUK)[5)] and rat pancreatic kallikrein (RPK)[6)] which were purified in our laboratory were also used in this investigation.

1) Part III: K. Kizuki, H. Moriya, and C. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 662 (1979).
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3) K. Kizuki, C. Moriwaki, Y. Hojima, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **24**, 1742 (1976).
4) H. Moriya, Y. Fukuoka, Y. Hojima, and C. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **26**, 3178 (1978).
5) Y. Matsuda, K. Miyazaki, H. Moriya, Y. Fujimoto, Y. Hojima, and C. Moriwaki, *J. Biochem.* (Tokyo), **80**, 671 (1976).
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Partially purified bovine, dog and rat kininogens were prepared by the procedures of Moriwaki and Schachter in which the kininogenases and the kininases in the plasma are inactivated by heat treatment.[7] These partially purified kininogens are called heated kininogens in this paper to distinguish them from kininogens in the fresh plasma (native kininogens). Shimeji kininase used in this experiment was purified as described in our previous paper.[3]

Kininase Activity Assay——This assay was carried out by the Magnus method using isolated guinea pig ileum.[3] One kininase unit is the amount of enzyme degrading 1 μg of synthetic bradykinin in 1 min at 30°, pH 7.4. The kininase activity of the stored enzyme solution was checked immediately before the following experiments.

Kinin Assay——An isolated rat uterus was suspended in a 10 ml bath of de Jalon's solution containing atropine (3×10^{-8} M) and mepyramine (5×10^{-8} M) and aerated at 30° under the tension of a 2 g weight. Synthetic bradykinin or assay sample was added to the bath with a polyethylene micropipette and the maximum contractile response of the uterus was recorded isotonically. Silicon-coated glassware or polyethylene containers were used to avoid the adsorption of bradykinin on glass.

Vasodilative Activity Assay——The vasodilative activity was determined by measuring the increase in arterial blood flow following the injection of samples into the femoral artery of an anesthetized dog according to the method of Moriya *et al.*[8]

Esterase Activity——The esterolytic activity of kallikreins towards BzArgOEt was determined at 25°, pH 8.0, according to the spectrophotometric method at 253 nm.[9] One esterase unit (EU) is the amount of enzyme hydrolyzing 1 μmol of BzArgOEt per min under these conditions.

Collection of Rat Plasma——Rats weighing 150—200 g were anesthetized with ether and 0.1 ml of heparin solution (1000 U/ml) was injected intravenously. The blood was collected from the carotid artery with a polyethylene cannula in a polyethylene test tube to avoid plasma prekallikrein activation. After that, it was centrifuged for 20 min at 4000 rpm and the separated plasma was used in the following experiments.

Determination of Kininogen Contents in Plasma——The high molecular weight kininogen (HMW-K) and low molecular weight kininogen (LMW-K) contents in plasma were determined by the following methods.

Method A: The procedures of Katori *et al.* were used with slight modifications.[10] HMW-K was determined by measuring the amount of kinin formed in plasma after the glass activation of plasma prekallikrein (plasma: glass beads, 1: 1, v/w) in the presence of 8-hydroxyquinoline (1 mg/ml), which is a plasma kininase inhibitor, because the plasma kallikrein specifically acts on the plasma HMW-K and liberates bradykinin. For the determination of LMW-K, the plasma was incubated in the absence of 8-hydroxyquinoline after the addition of glass beads until the HMW-K was completely consumed by the activated plasma kallikrein, then 8-hydroxyquinoline was added and maximum kinin liberation from the residual kininogen was obtained by addition of trypsin, which liberates bradykinin from both kininogens, to this plasma (1 mg/ml). SBTI was added to the sample solution (1 mg/ml) just before the kinin assay because trypsin itself sometimes contracts the rat uterus. The glass beads used were 50—100 mesh. The amount of kininogen was expressed as μg of bradykinin equivalent.

Method B: Shimeji kininase maintains kininase activity in the plasma for a fairly long time,[1] and is not inhibited by 8-hydroxyquinoline.[11] Thus, for the determination of kininogen contents in plasma collected from rats injected with Shimeji kininase, complete inactivation of Shimeji kininase in the plasma is necessary before the maximum kinin liberation in Method A. For this purpose, the plasma which contained Shimeji kininase was heated for 10 min at 58°. Shimeji kininase was so heat-labile that it was completely inactivated by this procedure.[11] HMW-K content in plasma which contained Shimeji kininase was determined by the following procedures. The plasma was heated, then 8-hydroxyquinoline and glass beads were added as in Method A. Maximum kinin liberation was then carried out by addition of an equal volume of non-heated rat plasma, which had been incubated in advance with glass beads for 30 min at 30° without 8-hydroxyquinoline, to this heated plasma. 8-Hydroxyquinoline was added to the later added plasma before incubation with the heated plasma. As the HMW-K in the later added plasma was completely consumed, the liberated kinin in the mixture of plasmas was estimated as that from HMW-K in the heated plasma. The kinin liberating enzyme would be the glass-activated plasma kallikrein in the later added plasma. For the determination of LMW-K, the plasma was incubated in the absence of 8-hydroxyquinoline after the addition of glass beads until the HMW-K was completely consumed by the activated plasma kallikrein, then it was heated and maximum kinin liberation was carried out by the addition of trypsin in the presence of 8-hydroxyquinoline.

7) C. Moriwaki and M. Schachter, *J. Physiol.*, **219**, 341 (1971).

8) *a*) H. Moriya, K. Yamazaki, and H. Fukushima, *J. Biochem.* (Tokyo), **58**, 201 (1965); *b*) C. Moriwaki, Y. Hojima, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **22**, 975 (1974).

9) G.W. Schwert and Y. Takenaka, *Biochim. Biophys. Acta*, **16**, 570 (1955).

10) M. Katori, S. Mineshita, and T. Shigei, "Advances in Experimental Medicine and Biology," Vol. 8, ed. by F. Sicuteri, M. Rocha e Silva and N. Back, Plenum Press, New York, 1970, pp. 273—282.

11) Part II: K. Kizuki, H. Moriya, and C. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 654 (1979).

Results

Effects on the Glandular Kallikreins

Shimeji kininase was separately incubated with hog PK, HUK and RPK for 20 hr and then remaining esterase activities were assayed. As shown in Table I, Shimeji kininase had no effect upon the esterase activity of hog PK. The esterase activities of HUK and RPK were somewhat reduced by this enzyme (about 15%). No esterase activity of Shimeji kininase itself was detected. Thus, this enzyme would hardly act on glandular kallikrein molecules. These results are consistent with the fact that glandular kallikreins such as HUK and hog PK are very resistant to the actions of various proteases.[12]

Table I. Effects of Shimeji Kininase on the Esterolytic Activities of the Glandular Kallikreins

Kallikrein	Remaining activity (EU/min/ml)	
	Control (A)	Sample (B)
Hog PK	1.35	1.33 (98.5)
HUK	0.14	0.12 (85.7)
RPK	2.28	1.93 (84.6)

Highly purified hog PK, HUK and RPK solutions (1 ml) were separately incubated with 1 ml of Shimeji kininase solution (40 kininase U/ml in 0.01 M phosphate buffer, pH 7.4) or 1 ml of the same buffer (control) for 20 hr at 20°, and then the esterolytic activities of these mixtures were determined. The activity was expressed as EU/min/ml of the above incubation mixture. The remaining activities of kallikreins are shown in parentheses as percentages (B/A×100).

Effects on the Heated Kininogens

Table II shows the effects of Shimeji kininase on heated dog, bovine and rat kininogens. These kininogens were separately incubated with the kininase at 30°, and after the indicated periods the mixtures were heated to destroy Shimeji kininase. Maximum kinin liberation from the remaining kininogen in the mixture was carried out by the addition of trypsin (1 mg/ml) in the presence of 8-hydroxyquinoline. Less remaining kininogen was found on longer incubation with the enzyme, so that this enzyme appeared to directly destroy the heated kininogens. Rat kininogen, however, was more resistant to this enzyme than the bovine kininogen.

Table II. Destruction of Heated Kininogens of Various Species by Shimeji Kininase

Kininogen	Residual kininogen (μg BKeq.)					
	0	0.5	1	2	3	15 hr
Bovine	2.36	1.80	1.04	0.56	0.16	0.00
Rat	1.20	1.20	1.18	1.12	1.04	0.00
Dog	1.40	—	—	—	—	0.00

The heated kininogen solutions (10 mg/ml in 0.01 M phosphate buffer, pH 7.4) 2 ml were separately incubated with 2 ml of Shimeji kininase solution (20 kininase U/ml in the same buffer) for the indicated periods at 30°. The amounts of kininogen remaining in the mixture were determined.

12) *a*) E. Werle and L. Maier, *Biochem. Z.*, **323**, 279 (1952); *b*) C.A.M. Sampaio and E.S. Prado, *Biochem. Pharmacol.*, **24**, 1438 (1975).

As Shimeji kininase consumed the heated kininogens, its kininogenase activity was examined by the Magnus method using various heated kininogens as substrates (Fig. 1). However, no kinin activity could be detected in the incubation mixture of Shimeji kininase and various kinds of kininogen (Fig. 1-C to -F). No kinin response was also detected at the varied incubation periods from 10 sec to 5 min. Fig. 1-G to -J shows the results of kinin liberation by hog PK and RPK from the same heated kininogens used in Fig. 1-C to -F. Kinin responses were detected in all these cases, so that the failure to detect kinin liberation in Fig. 1-C to -F was not due to the absence of kininogens in the substrate solution used. These results indicate that the destruction of the heated kininogens by Shimeji kininase (Table II) was not the result of kinin liberation from kininogen molecules by this enzyme; even if kinin was liberated from kininogen it would be rapidly inactivated by Shimeji kininase itself, since this enzyme is a potent kininase.

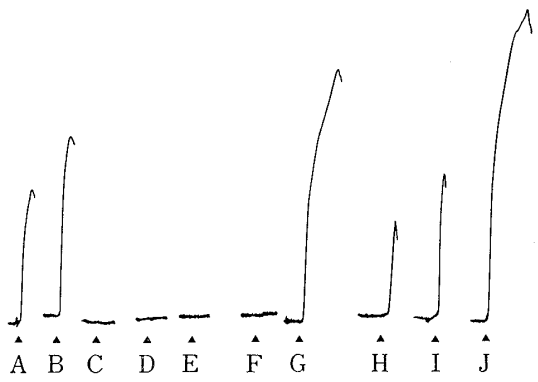

Fig. 1. Failure of Kinin Liberation from Heated Kininogens of Various Species by Shimeji Kininase

A and B, Bradykinin 2 and 4 ng, respectively. C, D, E and F, Shimeji kininase (100 μl) was incubated with 100 μl of heated bovine, hog, rat or human kininogen solution at 30°, and then 50 μl of the mixture was added to the organ bath after 1 min. G, H and I, The heated bovine, hog or human kininogen solution (100 μl) was incubated with 100 μl of hog PK (3.36 KU/ml) at 30°, and 50 μl was added to the organ bath after 1.5, 3 or 1 min, respectively. J, The heated rat kininogen solution (100 μl) was incubated with 100 μl of RPK (1.35 EU/ml) and 50 μl was assayed after 1 min.

Heated kininogens dissolved in 0.01 M phosphate buffer, pH 7.4, 50 mg/ml, and Shimeji kininase solution, 100 kininase U/ml, were used in these experiments.

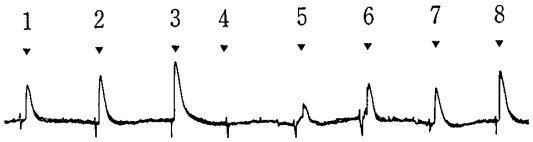

Fig. 2. The Vasodilative Activity of Shimeji Kininase

1 and 7, hog PK 0.005 KU; 2 and 8, hog PK 0.01 KU; 3, hog PK 0.02 KU; 4, Shimeji kininase 13 kininase U (480 kininase U/E_{280}); 5, Shimeji kininase 65 kininase U; 6, Shimeji kininase 130 kininase U.

The Vasodilative Activity of Shimeji Kininase

Figure 2 shows the results of vasodilative activity assay of Shimeji kininase in a dog. From the responses in Fig. 2, the vasodilative activity of this enzyme was calculated to be about 0.02 KU/E_{280} (KU: kallikrein unit). As compared with the activities of various kallikreins, this activity was negligible (see Discussion). Thus, it is confirmed that the destruction of kininogen shown in Table II was not the result of kinin liberation from kininogen by Shimeji kininase.

Effect on the Plasma Kininogen Contents

For the determination of kininogen contents in plasma which contained Shimeji kininase, heat treatment of the plasma was carried out (see Methods), so the effects of heating on the determination of kininogen contents were investigated. Fig. 3 shows typical time courses of kinin liberation from HMW-K by Methods A and B. The maximum kinin liberation by Method A was seen within 10 min, while 90 min or more was needed for maximum kinin liberation by Method B.

Table III shows the results of HMW-K and LMW-K determinations from four different normal rat plasma. The kininogen contents obtained by Method B were almost the same as those by Method A in each rat. This means that the heating which was carried out before the maximum kinin liberation did not have a serious effect upon the kininogen contents; HMW-K and LMW-K contents in plasma treated with Shimeji kininase could be determined even by Method B. However, 90 min or more incubation was necessary for maximum

kinin liberation from HMW-K in Method B. Rothschild and Gascon[13] reported that after the addition of glass powder to plasma, the TAME (*p*-tosylarginine methyl ester) esterase activity in the plasma rapidly increased, and the maximum activity was observed 1 min

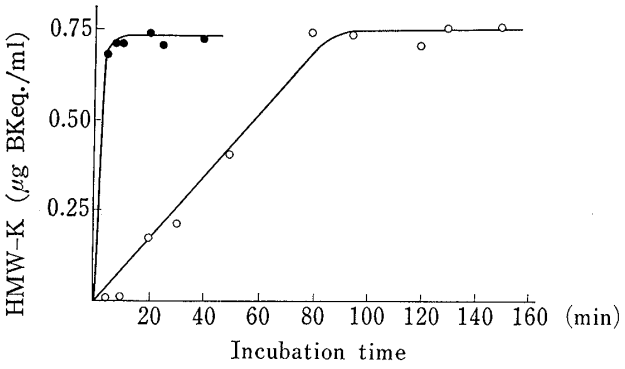

Fig. 3. Time Courses of Kinin Liberation from HMW-K

The plasma collected from a normal rat (rat No. 4 in Table III) was divided into halves, and kinin liberations from HMW-K were estimated by Methods A (●——●) and B (○——○).

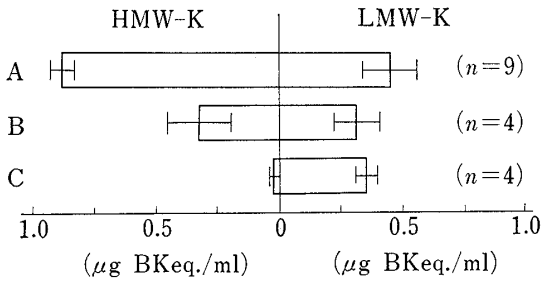

Fig. 4. Effects of Shimeji Kininase on Plasma Kininogen Contents

Shimeji kininase or saline was intravenously injected into rats from the femoral vein. After 20 min, the blood was collected from the carotid artery and the plasma was separated by centrifugation. A, saline 0.2 ml (control); B and C, Shimeji kininase 20 and 500 U administered, respectively. The kininogen contents in the control plasma were determined by Method A and the contents in the plasma collected from the rats given Shimeji kininase were determined by Method B. Figures in parentheses show numbers of rats.

Table III. HMW-K and LMW-K Contents in Rat Plasma determined by Two Methods

Rat No.	HMW-K (μg BKeq./ml)		LMW-K (μg BKeq./ml)	
	Method A	Method B	Method A	Method B
1	0.72	0.69	0.49	0.51
2	0.78	0.72	0.51	0.51
3	0.70	0.59	—	—
4	0.73	0.74	—	—

HMW-K and LMW-K contents in four normal rat plasma were determined by Methods A and B.

Table IV. Effect of Shimeji Kininase on Plasma LMW-K Content *in Vitro*

Incubation time (min)	LMW-K (μg BKeq./ml)
0	0.31 ± 0.02 ($n=4$)
30	0.27 ± 0.01 ($n=4$)
60	0.30 ± 0.01 ($n=4$)

Ten μl of Shimeji kininase solution (400 kininase U/ml) was added to 0.2 ml of fresh rat plasma and incubated at 30°. After 30 or 60 min, glass beads were added and incubated for 30 min at 30°. The mixture was then heated for 15 min at 58° and 8-hydroxyquinoline was added. After that, maximum kinin liberation was carried out by addition of 50 μl of RPK solution (6.77 EU/ml). Figures in parentheses show numbers of assays.

13) A.M. Rothschild and L.A. Gascon, *Nature* (London), **212**, 1364 (1966).

after the addition of glass powder. However, the activity gradually decreased and fell to half of the maximum after 12 min. Similar observations with cellulose sulfate[14] and carrageenin,[15] which cause plasma kallikrein activation, were reported by Rothschild and Di Rosa *et al.*, respectively. The gradual loss of activity is presumably due to inhibition of the activated kallikrein by the proteinase inhibitors in plasma, such as anti-trypsin and α_2-macroglobulin. Thus, the need for a longer incubation period in Method B may be due to the inhibition of the activated plasma kallikrein in the later added plasma by the proteinase inhibitors in it.

Based on the above observations, HMW-K and LMW-K contents in plasma collected 20 min after the intravenous injection of Shimeji kininase were separately determined (Fig. 4). Marked loss of HMW-K was caused by the injection of the enzyme and it almost disappeared in the plasma when 500 U of the enzyme was injected. On the other hand, the variations of LMW-K level were smaller.

Table IV shows the effects of Shimeji kininase on LMW-K content in the plasma *in vitro*. As shown in Table IV, the LMW-K level *in vitro* was confirmed to be unchanged even after the maximum kinin liberation by the administration of RPK.

It was concluded that Shimeji kininase hardly acts on the native LMW-K molecule, while HMW-K is consumed on incubation of rat plasma with the enzyme.

Activation of Plasma Prekallikrein by Shimeji Kininase

Figure 5 shows the activation of plasma prekallikrein by Shimeji kininase. The plasma prekallikrein was activated by contact with glass and liberated the kinin (Fig. 5-B and -C). Plasma which was incubated with 25 U of Shimeji kininase and heated for 10 min at 58° also caused the contraction of rat uterus on incubation with fresh rat plasma, and the contractile response became stronger on prolonger incubation (Fig. 5-E to -G). Plasma which was treated with 2.5 U of Shimeji kininase also caused contraction on incubation with fresh rat plasma (Fig. 5-H to -J), but the contractile responses were weak compared with those shown in Fig. 5-E to -G as judged from the necessary incubation time. On the other hand, plasma which was treated with saline instead of Shimeji kininase failed to liberate the kinin (Fig. 5-D). These results indicate that the contractile responses (E to J) in Fig. 5 were due to a substance which was enzymatically liberated in the plasma by the action of Shimeji kininase. The liberation of this substance, however, was not due to the direct action of the enzyme itself because the enzyme was completely inactivated by heating. On the other hand, intravenous injection of Shimeji kininase caused specific consumption of plasma HMW-K (Fig. 4). Thus, these contractile responses can be explained by kinin liberation as a result of plasma prekallikrein activation by Shimeji kininase, because plasma kallikrein specifically acts on plasma HMW-K and causes HMW-K consumption. This view is supported by the following observations. First, heat treatment of rat plasma did not cause plasma prekallikrein activation (Fig. 6-B). Second, the heated plasma did not liberate kinin on addition of glass beads (Fig. 6-C), *i.e.*, the plasma prekallikrein activating system in the plasma was destroyed by heating and plasma prekallikrein activation could not take place after the heat treatment. Third, plasma which was heated after the activation of plasma prekallikrein by glass still liberated kinin on incubation with fresh plasma (Fig. 6-F to -I). These responses were due to kinin liberated from HMW-K in the later added fresh plasma, because heated plasma contained neither HMW-K nor free kinin. The absence of HMW-K and free kinin in the heated plasma was confirmed by the results in D and E. Namely, glass-activated kallikrein liberated the kinin from HMW-K but the liberated kinin was inactivated by plasma kininases (D). Complete HMW-K consumption was obvious, as

14) A.M. Rothschild, *Brit. J. Pharmacol.*, 33, 501 (1968).
15) M. Di Rosa and L. Sorrentino, *Brit. J. Pharmacol.*, 38, 214 (1970).

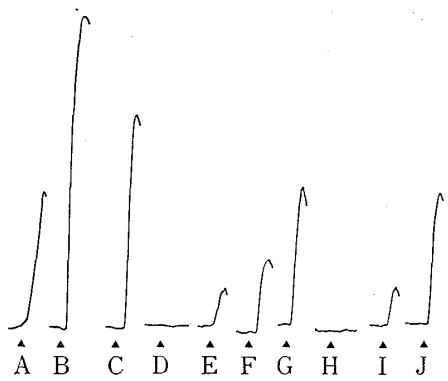

Fig. 5. Kinin Liberation from Rat Kininogen by Shimeji Kininase-Treated Rat Plasma

A, Bradykinin 1 ng. B and C, Rat plasma was incubated with glass beads for 20 min at 30° in the presence of 8-hydroxyquinoline, and then 20 or 5 μl was added to the organ bath, respectively. E, F and G, 100 μl of Shimeji kininase solution (250 kininase U/ml) was added to 1 ml of fresh rat plasma and incubated for 30 min at 30°. After heat treatment, it was incubated with 1 ml of fresh rat plasma in the presence of 8-hydroxyquinoline at 30°. A part of this mixture (20 μl) was assayed after 5, 10 and 15 min incubation. H, I and J, Ten μl of Shimeji kininase solution was added to 1 ml of rat plasma and procedures E to G were carried out. A part of this mixture (20 μl) was assayed after 5, 15 or 25 min incubation. D, Saline (100 μl) was added to 1 ml of rat plasma instead of Shimeji kininase and procedures E to G were carried out. Twenty μl of the incubation mixture was added to the organ bath after 20 min.

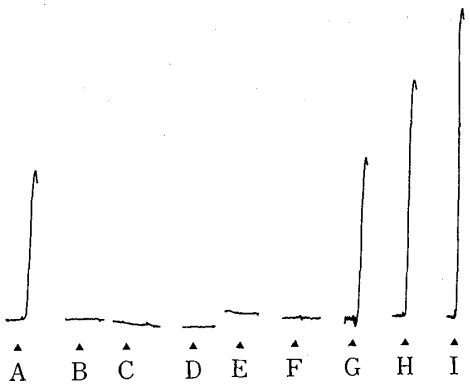

Fig. 6. Effect of Heating on the Kininogenase Activity of the Glass-Activated Plasma Kallikrein

A, Bradykinin 2 ng. B, Fresh rat plasma which contained 8-hydroxyquinoline was heated for 10 min at 58°. After cooling with ice, 20 μl was added to the organ bath. C, Glass beads were added to B and incubated for 10 min at 30°, then 20 μl was assayed. D, Glass beads were added to fresh rat plasma without 8-hydroxyquinoline and incubated for 15 min at 30°. It was heated for 10 min at 58° and 20 μl was assayed. E, 8-Hydroxyquinoline was added to the above plasma D and incubated at 30°. After 10 min, 20 μl was assayed. F, G, H and I, Fresh rat plasma which contained 8-hydroxyquinoline was added to E in a ratio of 1:1 (v/v). After 5, 10, 15 and 20 min at 30°, 20 μl of this incubation mixture was assayed.

shown in E, because no kinin response could be detected even after the inhibition of plasma kininases by 8-hydroxyquinoline. The kinin-liberating activity of the heated plasma, however, was far less than that of non-heated plasma. These observations mean that a considerable amount of plasma kallikrein which had been activated in advance was inactivated by this heating and perhaps by proteinase inhibitors in plasma but a part of its activity remained. Hence, the contractile responses (E to J) in Fig. 5 would be explained in terms of kinin liberation by plasma kallikrein which was activated by Shimeji kininase.

Discussion

The effects of Shimeji kininase on the kallikrein–kinin system in addition to kinin destruction were studied. This enzyme gradually consumed the heated kininogens of various species (Table II). Thus, this enzyme may directly destroy the kininogens *in vivo*, because it retains kininase activity in the rat vascular system for a fairly long time.[1] However, there was no significant difference in the level of LMW-K in the rat after intravenous injection of 500 U of Shimeji kininase (Fig. 4), and the LMW-K content in a mixture of Shimeji kininase and fresh rat plasma was not altered even after 1 hr (Table IV). Thus, Shimeji kininase hydrolyzed the native LMW-K molecule very slowly or not at all. This observation, however, seemed to be in conflict with the finding that the present enzyme gradually consumed heated kininogens of various species, which were mixtures of HMW-K and LMW-K (Table II). One possible explanation for this is denaturation of the kininogen molecules, *i.e.*, heat-denatured kininogen (heated kininogen) may be more easily hydrolyzed than the native material. This explanation is supported by the observations that proteolytic

enzymes, such as trypsin, papain, leucine aminopeptidase, subtilisin and so on, generally hydrolyze denatured proteins more easily than native proteins.[16]

On the other hand, the direct action of Shimeji kininase on HMW-K *in vivo* was obscure as yet because the disappearance of HMW-K which was considered to be due to the plasma prekallikrein activation was taken place by Shimeji kininase.

The vasodilative activity of Shimeji kininase was 0.02 KU/E_{280}. The vasodilative activity of kallikrein is usually proportional to the kinin-forming activity[8b] and various kinds of purified kallikrein had activities of several hundred to a thousand KU/mg or KU/E_{280}. For example, hog PK,[4] HUK[5] and human salivary kallikrein[17] have activities of 1430 KU/mg, 200 and 1117 KU/E_{280}, respectively. On the other hand, plasma contains activity amounting to 1—10 KU/ml.[18] Some of the most potent kininases, ficins, have activities of 0.28 to 2.8 KU/mg.[19] This enzyme liberated a kinin-like substance from heated horse plasma, but this substance was rapidly inactivated by ficin itself.[20] As compared with these values, that of Shimeji kininase is extremely small and the kinin liberation was not detectable by the Magnus method. Thus, this enzyme was considered not to have kininogenase activity.

The present enzyme had a remarkable action on the kallikrein–kinin system, *i.e.*, this enzyme activated plasma prekallikrein and caused the specific consumption of plasma HMW-K. Plasma prekallikrein activation by Shimeji kininase was qualitatively confirmed by the kinin liberation (Fig. 5). Katori *et al.* reported that bromelain, which has potent kininase activity, activated plasma prekallikrein and caused the depletion of plasma HMW-K, but papain, which is also potent kininase, did not have such an effect.[10] Recently, they showed that plasma prekallikrein activation by bromelain was due to the activation of pre-Hageman factor.[21] The mechanism in the case of Shimeji kininase is not yet clear.

In the previous paper, we reported that intravenous injection of high doses of Shimeji kininase (500 U or more) in the rat caused a slight but continuous fall of arterial blood pressure; this fall of blood pressure could not be explained by the direct action of Shimeji kininase itself, because the effect recovered to the normal level within 10—15 min, though Shimeji kininase maintained its activity in the vascular system even after that period.[1] At present, the fall of blood pressure induced by Shimeji kininase seems explicable in terms of the activation of plasma prekallikrein. Namely, activated plasma kallikrein continuously liberated the kinin from HMW-K and the liberated kinin caused the hypotension. On the other hand, Shimeji kininase is a potent kininase, so that most of the kinin which was liberated by plasma kallikrein was rapidly inactivated by the enzyme itself and partially by plasma kininases. Consequently, the fall of blood pressure induced by the intravenous injection of Shimeji kininase was small and short-lived.

Thus, Shimeji kininase had an effect on the kallikrein–kinin system in addition to the destruction of kinin, *i.e.*, plasma prekallikrein activation. However, Shimeji kininase itself would rapidly destroy even the kinin generated in the plasma as a result of plasma prekallikrein activation, so its use as a blocker of kinin action in the body may be feasible in experiments on the kallikrein–kinin system.

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