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Kinin Inactivating Enzyme from Mushroom *Tricholoma conglobatum*. III. Stability in the Rat Body and Kinin Suppression *in Vivo*[1]

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The suppression of kinin action in the body by Shimeji kininase was studied in rat. This enzyme was purified from a mushroom *Tricholoma conglobatum* (Shimeji, in Japanese) and had the highest kinin inactivating activity among the ever known kininases from plants. The enzyme was stable in rat plasma for a long time *in vitro*. The enzyme also maintained the activity in the plasma *in vivo* and the loss of activity from the rat vascular system was too small even 1 hr after the intravenous injection of it.

This enzyme markedly suppressed the hypotensive effect of bradykinin and the capillary permeability increase induced by bradykinin. In contrast with this, the hypotension caused by acetylcholine and the capillary permeability increase by histamine and serotonin were not suppressed by this enzyme.

From these observation, this enzyme was considered to be able to block the kinin action in the body for a certain period due to the rapid kinin destruction by it.

The enzyme also had the potent anti-inflammatory activity on the rat paw edema induced by carrageenin.

Keywords——kininase from mushroom *Tricholoma conglobatum*; kinin destruction; suppression of kinin action; capillary permeability; anti-inflammation

In order to clarify the physiological and pathological significances of kallikrein-kinin system in the body, the agents which affect on this system, such as the specific kallikrein inhibitor, the strong anti-kinin substance and the potent kininase, are seemed to be useful because the roles of this system should be revealed in the experiments in which the system is specifically blocked. Many studies have been attempted for this purpose, for example, the synthesis of several bradykinin analogs expecting competitive inhibition of bradykinin action[3] or the search of the anti-kinin substance from various materials,[4] but these attempts were seemed not to be specifically advantageous.

The authors have been working on the potent kinin inactivating enzyme, named as Shimeji kininase, which was purified from a mushroom *Tricholoma conglobatum* (Shimeji, in Japanese) for the purpose of blocking the kinin action in the body.[5]

In the present paper, the authors describe about the stability of this enzyme in the rat body and the suppression of kinin action by it in rat. The details of the purification procedures and some enzymatic properties of this enzyme were already reported.[1,5]

Materials and Methods

Materials——Bradykinin was supplied from Protein Research Foundation (Osaka) and Sephadex G-200 obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Histamine dichloride, serotonine creatinine

1) Part II: K. Kizuki, H. Moriya, and C. Moriwaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 654 (1979).
2) Location: *Ichigaya Funagawaracho, Shinjuku-ku, Tokyo.*
3) J.M. Stewart and D.W. Woolley, "International Symposium on Vaso-Active Polypeptides: Bradykinin and Related Kinins," ed. by M. Rocha e Silva and H.A. Rothschild, III. International Pharmacological Congress and Soc. Bras. Farmacologia e Terapeutica Expêrimental, São Paulo, 1967, p. 7.
4) H. Nagase, Y. Hojima, C. Moriwaki, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **23**, 971 (1975).
5) K. Kizuki, C. Moriwaki, Y. Hojima, and H. Moriya, *Chem. Pharm. Bull.* (Tokyo), **24**, 1742 (1976).

sulfate and acetylcholine chloride were products from E. Merk (Darmstadt, Germany), Nakarai Chemicals, Ltd. (Kyoto) and Tokyo Kasei Kogyo Co. (Tokyo), respectively. λ-Carrageenin from Pasco International Co. (Tokyo), sodium pentobarbital from Pitman-Moore, Ltd. (Washington, N.J., U.S.A.) and Evans' blue from Tokyo Kasei Kogyo Co. were also used in this investigation. Shimeji kininase was purified as described in our previous paper.[5] Kininase activity of the preserved enzyme solution was checked up just before the following experiments.

Kininase Activity Assay——This assay was carried out by Magnus method using guinea pig ileum.[5] One kininase unit was the amount of enzyme that could degrade 1 μg of synthetic bradykinin per min at 30°, pH 7.4.

Arterial Blood Pressure Assay——The arterial blood pressure was recorded by the modified method of Grez *et al.*[6] Wister albino rats weighing 200—250 g were anesthetized with sodium pentobarbital in a dose of 50 mg/kg of body weight and the polyethylene cannulas were introduced to the left carotid arteries. The cannula tips were advanced to the arcus aorta and 0.15 ml of heparin solution (1000 U/ml) was injected through these cannulas. On the other hand, another cannulas in which heparin solution was filled were introduced to the left femoral arteries and connected to the pressure transducer (model MPU-0.5, Nihon Koden, Japan). Samples, except Shimeji kininase, were injected through the former cannulas. Shimeji kininase was injected from the right femoral vein with a syringe.

Sephadex G-200 Gel Filtration——One ml of the fresh rat plasma which was collected after the intravenous administration of Shimeji kininase was gel filtered with Sephadex G-200 column (1.5×88 cm) equilibrated with 0.01 M phosphate buffer, pH 7.4 containing 0.9% NaCl at 4°. The column was developed with the same buffer and the eluate was collected in 2.1 ml per tube at a flow rate of 10.5 ml/hr.

Capillary Permeability——The rats weighing 140—160 g were anesthetized intraperitoneally with sodium pentobarbital and clipped off their venter fur by hair clippers from the venter cranialis to the venter caudalis. Then, the rats were intravenously injected with 5% (w/v) Evans' blue in saline *via* the femoral vein in a dose of 1 ml/kg of body weight. Five minutes later intradermal injections of the permeability increasing agents, bradykinin (10 μg/ml), histamine (10 μg/ml) and serotonin (5 μg/ml) in saline, were made in 0.1 ml volumes. After 30 min, the rats were exsanguinated and Evans' blue leaked into the skin was extracted and determined according to the method of Shimomura and Fukushima.[7] Shimeji kininase, which was dialyzed against saline for 15 hr at 4° was intravenously administered 5 min before the injection of Evans' blue or intraperitoneally administered 15 min before the injection of Evans' blue.

Rat Paw Edema——The rats weighing 160—170 g were used. The rats were slightly anesthetized with ether and 0.1 ml of 1% (w/v) carrageenin or 15% of fresh egg white solution were injected beneath the plantar aponeurosis of the right hind paws. The same volume of saline was administered to the left hind paw at the same time. Edema volume was the volume of right paw minus that of left one at each time. Shimeji kininase was intraperitoneally administered 20 min before the injection of carrageenin or egg white. The volume of the rat hind paws were measured by the modified apparatus of Harris and Spencer.[8]

Results

Stability in the Rat Plasma

Figure 1 showed the kinin destruction by the incubation mixture of Shimeji kininase and rat plasma in the presence of 8-hydroxyquinoline. Under this condition, rat plasma itself did not destroy bradykinin due to the complete inhibition of kininases existed in rat plasma by 8-hydroxyquinoline (the open square in Fig. 1). On the other hand, the incubation mixture of Shimeji kininase and rat plasma destroyed bradykinin even in the presence of 8-hydroxyquinoline (the closed circle and square in Fig. 1). The incubation mixture of Shimeji kininase and saline which was the control of the above two samples of course destoryed bradykinin in the presence of 8-hydroxyquinoline (the open circle in Fig. 1). Accordingly, the destruction of bradykinin shown in Fig. 1 was based on Shimeji kininase itself. The kininase activity of these incubation solutions, *i.e.*, the incubation mixture of Shimeji kininase and saline (the open circle in Fig. 1), the incubation mixtures of Shimeji kininase and rat plasma for 1 and 3 hr (the closed circle and square in Fig. 1, respectively), were calculated to be 2.21, 1.90 and 1.65 kininase U/ml, respectively, from the amounts of inactivated bradykinin (calculated from the mean of the amounts of inactivated bradykinin at 10, 20 and

6) M.M. Grez, M.S.M. Grez, and G. Peters, *Eur. J. Pharmacol.*, **29**, 35 (1974).
7) K. Shimomura and T. Fukushima, *J. Pharm. Pharmacol.*, **24**, 837 (1972).
8) J.M. Harris and P.S.J. Spencer, *J. Pharm. Pharmacol.*, **14**, 464 (1962).

30 min incubation periods). The latter two values were 86 and 75% of the first one. Thus, this enzyme was considered to maintain the kininase activity in the rat plasma for a fairly long time without much loss of activity.

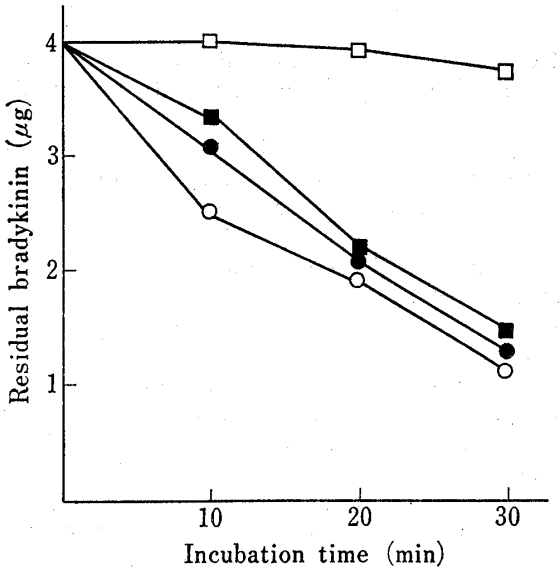

Fig. 1. Kininase Activity of Shimeji Kininase after Treatment with Rat Plasma

Shimeji kininase solution (0.1 ml) was added to 1.5 ml of fresh rat plasma and incubated for 1 and 3 hr at 30°. Then, kinin inactivating activity of these mixture was assayed by the following method. After the addition of 8-hydroxyquinoline to the mixture (final concentration, 1 mg/ml), 50 μl of this mixture was incubated with 2 ml of synthetic bradykinin solution (2 μg/ml in 0.02 M phosphate buffer, pH 7.4 containing 1 mg/ml of 8-hydroxyquinoline). After a certain period (abscissa in Fig. 1), residual bradykinin was assayed by Magnus method. The ordinate showed the residual bradykinin in the above incubation mixture.

—●— and —■—: Incubation with rat plasma were carried out for 1 and 3 hr, respectively, then assayed after incubation with bradykinin; —○—: Shimeji kininase was incubated with saline for 3 hr instead of rat plasma, then assayed after incubation with bradykinin; —□—: rat plasma (50 μl) was incubated with bradykinin under the presence of 8-hydroxyquinoline.

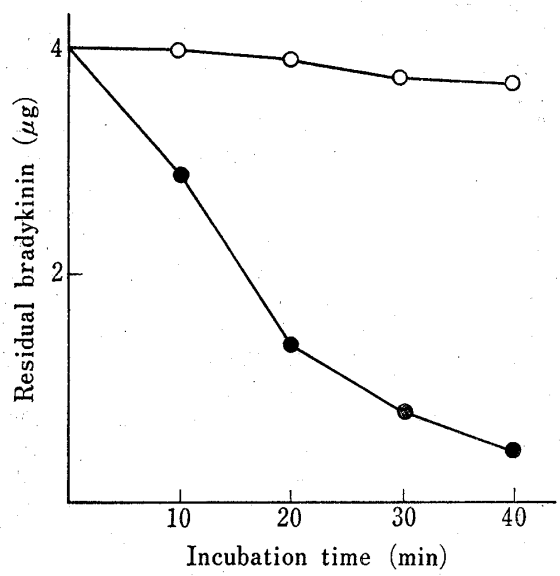

Fig. 2. Kininase Activity in Rat Plasma after the Intravenous Administration of Shimeji Kininase

Shimeji kininase (30 kininase U) was intravenously injected to rat. After 20 min, the blood was collected from the carotid artery with the polyethylene cannula and centrifuged for 20 min at 4000 rpm. Then, 50 μl of the separated plasma was incubated with 2 ml of bradykinin solution (2 μg/ml) for the indicated periods under the presence of 8-hydroxyquinoline and the residual bradykinin was assayed by Magnus mehod (—●—). —○—: 50 μl of the rat plasma which was collected after 20 min an intravenous injection of saline was incubated with bradykinin solution under the presence of 8-hydroxyquinoline.

Stability of Shimeji Kininase in the Rat Body

The rat plasma collected after an intravenous injection of Shimeji kininase still retained the kininase activity which was based on Shimeji kininase (Fig. 2). However, the recovery against the total administered kininase activity could not be calculated because the dilution problem of this enzyme into the rat body could not be estimated. Fig. 3 showed the chromatographical analysis of the kininase activity of Shimeji kininase in rat plasma by Sephadex G-200 gel filtration. A in Fig. 3 was the elution pattern of rat plasma itself. Kininases in the plasma (kininase I and II) were eluted out in fractions 21 to 26 as indicated. On the contrary, Shimeji kininase was eluted out in fractions 30 to 42 (Fig. 3-B). C and D in Fig. 3 were the results of rat plasma which were collected at 5 min and 1 hr after the intravenous administrations of Shimeji kininase. In both cases, kininase activity, which was assayed under the presence of 8-hydroxyquinoline (1 mg/ml), was only detected in fractions 30 to 42 which were the same position as authentic Shimeji kininase (B). The amount of the eluted kininase activity in Fig. 3-D was 85% of that of the activity observed in Fig. 3-C. These results mean that Shimeji kininase was hardly inhibited by plasma proteinase inhibitors and did not instantly disappear in the vascular system.

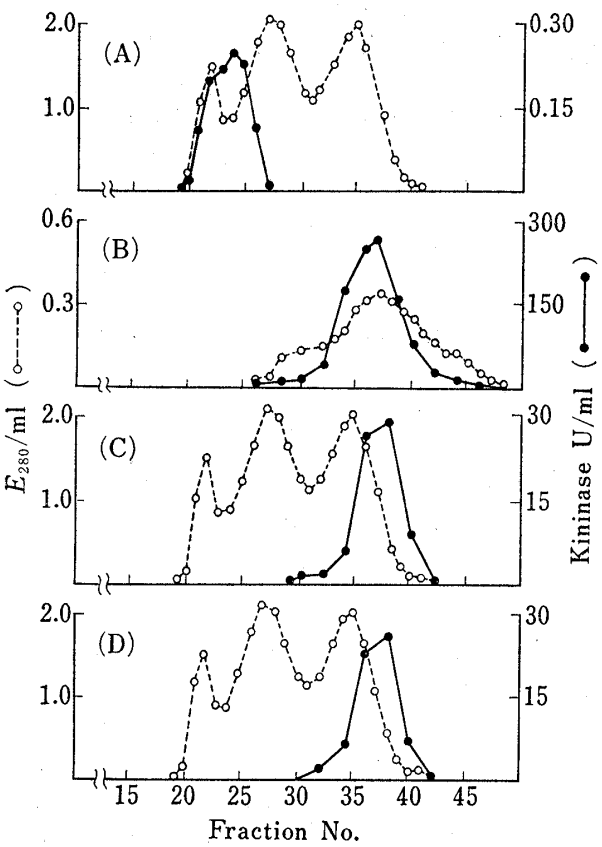

Fig. 3. Sephadex G-200 Gel Filtration of Rat Plasma after Intravenous Injection of Shimeji Kininase

(A): Fresh rat plasma; (B): Shimeji kininase (3000 U/ml); (C) and (D): Fresh rat plasma which was collected after 5 min and 1 hr intravenous injection of 3000 U of Shimeji kininase (rat weight: 230 g), respectively. Kininase activities were assayed under the presence (C and D) or absence (A and B) of 8-hydroxyquinoline.

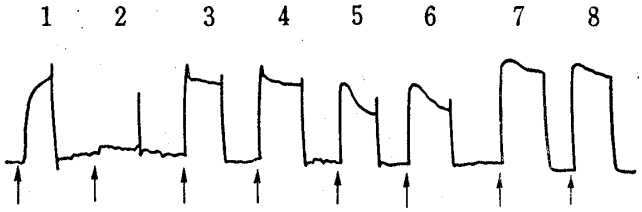

Fig. 4. Effects of Shimeji Kininase on Bradykinin, Histamine, Serotonin and Acetylcholine

Bradykinin, histamine, serotonin and acetylcholine were dissolved in 0.05 M phosphate buffer, pH 7.4 at a concentration of 1, 1, 2 and 1 μg/ml, respectively and 0.15 ml of them were separately incubated with 0.15 ml of Shimeji kininase solution (2 kininase U/ml) at 30°. Then, remaining activities of them in 0.2 ml of these mixture were assayed.

(1): bradykinin 100 ng; (2): bradykinin 100 ng+Shimeji kininase, incubated for 3 min; (3): histamine 100 ng; (4): histamine 100 ng+Shimeji kininase, incubated for 15 min; (5): serotonin 200 ng; (6): serotonin 200 ng+Shimeji kininase, incubated for 15 min; (7): acetylcholine 100 ng; (8): acetylcholine 100 ng+Shimeji kininase, incubated for 15 min.

Effects of Shimeji Kininase on the Actions of Histamine, Serotonin and Acetylcholine

Shimeji kininase was separately incubated with bradykinin, histamine, serotonin, and acetylcholine then residual activities of them were assayed by the contraction of isolated guinea pig ileum (Fig. 4). The same amount of Shimeji kininase which completely inactivated 100 ng of bradykinin within 3 min caused no change on the contractile responces of histamine (100 ng), serotonin (200 ng), and acetylcholine (100 ng) even after 15 min incubation.

Effect of Shimeji Kininase on Hypotensive Action of Bradykinin on Arterial Blood Pressure

Figure 5 and 6 showed the effect of Shimeji kininase on arterial blood pressure and the suppression of hypotensive effect of bradykinin by this enzyme. Intravenous administration

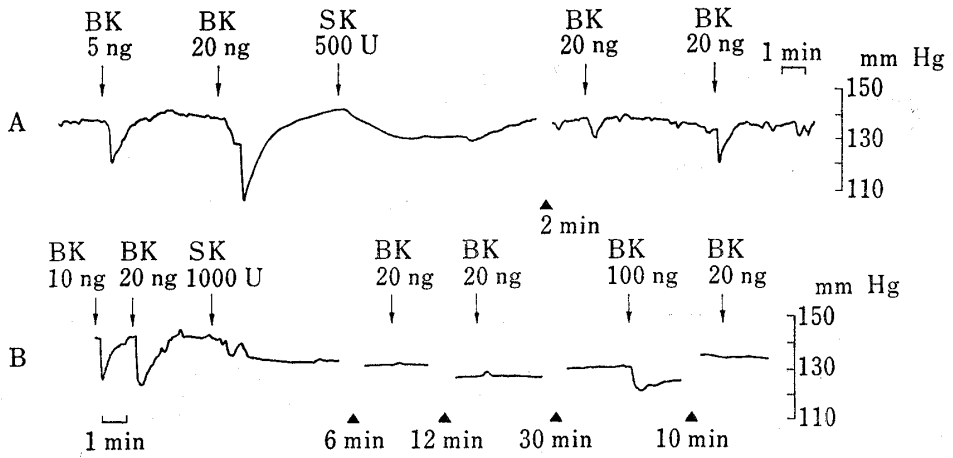

Fig. 5. Effect of Shimeji Kininase on Arterial Blood Pressure of Rat and Suppression of the Hypotensive Effect of Bradykinin by It

Shimeji kininase, 500 (A) and 1000 kininase U (B) were intravenously injected in rat. BK: bradykinin; SK: Shimeji kininase.

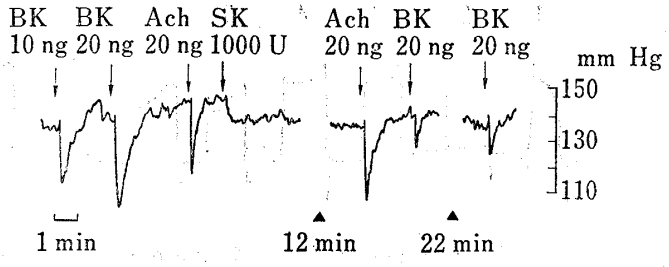

Fig. 6. Effect of Shimeji Kininase on the Hypotensive Effects of Bradykinin and Acetylcholine

BK: bradykinin; Ach: acetylcholine; SK: Shimeji kininase.

of 500 U of the enzyme immediately led to a slight decrease in blood pressure but the blood pressure restored to the normal level within 10—15 min (Fig. 5-A). In the case of 1000 U injection, the drop of blood pressure, which also gradually restored, continued for some more period (Fig. 5-B).

In both cases, the hypotensive response of bradykinin was markedly suppressed after the intravenous administration of Shimeji kininase and this effect lasted for more than 1 hr. On the other hand, hypotensive effect of acetylcholine was not affected by the intravenous administration of Shimeji kininase (Fig. 6). The low dose (20 kininase U) of Shimeji kininase caused no detectable influence of the kinin suppression upon the arterial blood pressure.

Anti-Inflammatory Effects

Kinins and biogenic amines, histamine and serotonin, considered to be the chemical mediators in inflammation and it was observed that the intradermal injection of them induced a capillary permeability increase in mammals.

Table I showed the inhibitory effect of Shimeji kininase on vascular permeability increase induced by 1 μg of bradykinin. Dye leakage at the position of bradykinin injected site was significantly suppressed by intravenous injection of 20 U or more Shimeji kininase, or by

Table I. Inhibitory Effect of Shimeji Kininase on Capillary Permeability Increase induced by Bradykinin (1 μg)

	Dose and route	Leaked Evens' blue E_{615}/ml	(n)	Inhibition %
Control: saline	0.1 ml *i.v.*	0.335±0.016	(16)	
Shimeji kininase	5 U *i.v.*	0.296±0.015	(18)	11.6
	20 U *i.v.*	0.195±0.015	(18)	41.8
	80 U *i.v.*	0.116±0.021	(16)	65.4
Control: saline	0.5 ml *i.p.*	0.223±0.009	(28)	
Shimeji kininase	360 U *i.p.*	0.184±0.010	(36)	17.5
	720 U *i.p.*	0.138±0.007	(18)	38.1
	1300 U *i.p.*	0.138±0.008	(16)	38.1

Intravenous (*i.v.*) and intraperitoneal (*i.p.*) injections of Shimeji kininase were made in 0.1 ml and 0.5 ml volumes, respectively. Test number of experiments was indicated as *n*.

Table II. Effect of Shimeji Kininase on Capillary Permeability Increase induced by Histamine and Serotonin

	Dose and route	Leaked Evans' blue E_{615}/ml	(n)	Inhibition %
Histamine (1 μg)				
Control: saline	0.2 ml *i.v.*	0.289±0.021	(18)	
Shimeji kininase	240 U *i.v.*	0.277±0.018	(23)	4.2
Serotonin (0.5 μg)				
Control: saline	0.2 ml *i.v.*	0.740±0.064	(18)	
Shimeji kininase	240 U *i.v.*	0.644±0.031	(19)	13.0

Intravenous (*i.v.*) injection of Shimeji kininase was made in 0.2 ml volume. Test number of experiments was indicated as *n*.

intraperitoneal injection of 720 U of it. In contrast with this observation, the vascular permeability increase induced by 1 μg of histamine and 0.5 μg of serotonin was hardly suppressed even by intravenous administration of 240 U of Shimeji kininase (Table II).

Besides the antagonization to bradykinin action on the vascular permeability, this enzyme inhibited the rat paw edema formation by carrageenin (Fig. 7). The paw swelling produced in the first 1.5 hr was not suppressed by the intraperitoneal administration of 370 U of Shimeji kininase but suppression of paw swelling became remarkable from 3 hr, and the edema was more strongly suppressed by high dose of Shimeji kininase.

On the other hand, edema formation by egg white was somewhat suppressed by this enzyme. However, dose dependency of Shimeji kininase on this edema was not observed (Fig. 8).

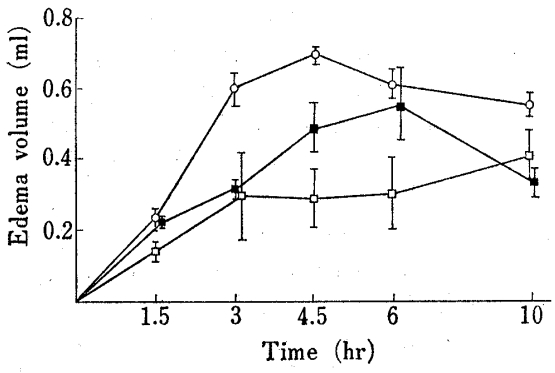

Fig. 7. Inhibition of Carrageenin-Induced Edema in the Rat's Paw by Shimeji Kininase

Before the injection of carrageenin, 0.5 ml of saline (—○—), 370 kininase U of Shimeji kininase (—■—) and 1100 kininase U of Shimeji kininase (—□—) were intraperitoneally administered. Five animals were employed in each group.

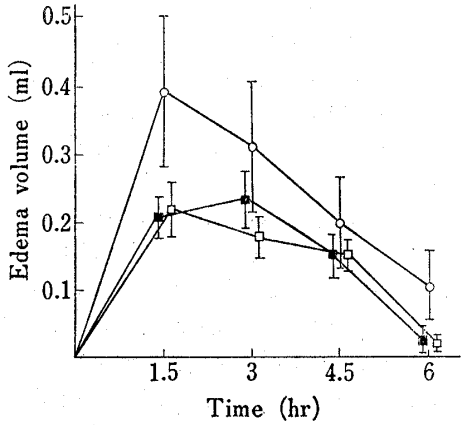

Fig. 8. Inhibition of Egg White-induced Edema in the Rat's Paw by Shimeji Kininase

Before the injection of egg white, 0.5 ml of saline (—○—), 420 kininase U of Shimeji kininase (—■—) and 840 kininase U of Shimeji kininase (—□—) were intraperitoneally administered. Five animals were employed in each group.

Discussion

Much progress has been made in the study of the roles of physiologically active substances, such as histamine, serotonin, and acetylcholine, with respect to the advanced study of specific antagonists for them. However, at the present time we possess no specific antagonist to kinins and have only the compounds which have somewhat antagonistic properties to kinins. These compounds also antagonized histamine, acetylcholine, and so on at the same level of concentrations that antagonized bradykinin. Besides the search for the specific antagonist to kinins, some attempts to block the kinin action in the body have been tried by using some proteolytic enzymes, such as carboxypeptidase B (CPase B)[9] and the kininase from the microbe *Pseudomonas aeruginosa* (*Ps. aer.*).[10] These attempts, however, were not sufficiently accomplished because of the rapid disappearance of their activities in the vascular system. One of the difficulties to search for the roles of kallikrein-kinin system in the body would lie in these points. CPase B and the kininase from *Ps. aer.* can suppress the hypotensive effect of bradykinin when they were given intravenously to the experimental animals.[9,10b] However, their half-life in circulation in the body was very short. The half-

9) *a*) E.G. Erdös, J.R. Wohler, and M.I. Levine, *J. Pharmacol. exp. Ther.*, **142**, 327 (1963); *b*) E.G. Erdös and H.Y.T. Yang, "Handbook of Experimental Pharmacology," Vol. XXV, ed. by E.G. Erdös, Springer-Verlag, Berlin-Heiderberg-New York, 1970, pp. 289—323.

10) *a*) H.E. Rugstad, *Br. J. Pharmacol.*, **30**, 134 (1976); *b*) *Idem, ibid.*, **30**, 425 (1967).

life of CPase B was 30 min in cat and 17 min in rabbit[9] and that of the kininase from *Ps. aer.* was about 20 min in rat.[10b] As compared with these enzymes, the half-life of Shimeji kininase seems to be fairly long as judged from the results of the chromatographical analysis by Sephadex G-200 (Fig. 3). According to Rugstad, the rapid disappearance of intravenously injected kininase from *Ps. aer.* from the vascular circulation would be due to the passage of this enzyme molecule throughout the capillary walls, because any disappearance of its activity could not be detected even after *in vitro* incubation with blood for 24 hr.[10a] With regard to this consideration, the molecular weight of Shimeji kininase was 6.6×10^4 [1] while that of the kininase from *Ps. aer.* was 5×10^4 [10b] and CPase B, 3.4×10^4.[11] Thus, this consideration would be one of the reasonable explanation as a size of molecule for the long half-life of Shimeji kininase.

Judging from the remarkable stability of Shimeji kininase in the rat plasma *in vitro* (Fig. 1), this enzyme might be thought not to be inhibited by proteinase inhibitors in plasma. α_2-Macroglobuline (α_2M), one of the main proteinase inhibitors in plasma, is usually capable of binding a considerable number of endopeptidases from different origin and α_2M bound enzymes has almost complete nothing of its activity towards native proteins, but α_2M bound enzymes still maintain the activity to hydrolyze low molecular weight substrates such as angiotensin, vasopressin and synthetic substrates as rapidly as free enzymes.[12] Angiotensin and vasopressin are the octapeptides and bradykinin is the nonapeptide. Accordingly, it would be possibly assumed that α_2M-Shimeji kininase complex would be formed in the plasma and this complex still hydrolyzed the kinins. One of the ways to check up this assumption was to observe its chromatographical behaviour on Sephadex G-200 gel filtration. In this analysis, α_2M and α_2M-enzyme complex should be eluted out at void volume because the molecular weight of α_2M is very large. So that, if α_2M-Shimeji kininase complex would be formed in plasma, kininase activity should be detected at the void volume and the original kininase activity which was eluted out in fractions 30 to 42 in Fig. 3-B should be decreased. The kininase activity, however, was not detected at void volume and eluted out at the same position as authentic Shimeji kininase (Fig. 3-C and -D). Accordingly, the kininase activity in the plasma after the intravenous injection of Shimeji kininase was not based on α_2M-Shimeji kininase complex, and Shimeji kininase would be existed as a free form in the vascular system. Besides the differentiation of the molecular weight, the long half-life of Shimeji kininase in the vascular system could be explained from the above mentioned matter because the exogenious proteinases bound to α_2M are rapidly cleared from the circulation.[13]

Our present enzyme markedly suppressed the hypotensive and the capillary permeability increasing effects induced by bradykinin, but did not affect on those induced by acetylcholine, histamine and serotonin whose biological activities were not affected by Shimeji kininase. Accordingly, the suppression of the kinin action in the body was explained by rapid kinin destruction by Shimeji kininase.

In addition to the marked suppression of the hypotensive effect of bradykinin, this enzyme had the potent anti-inflammatory effect on edema formation induced by carrageenin. Rosa and Willoughby showed that the edema induced by carrageenin is mediated by histamine and serotonin during the first 1 hr, after which the increase of vascular permeability is maintained by kinin released up to 2.5 hr.[14] Failure of carrageenin edema suppression by Shimeji

11) J.E. Fork, "Methods in Enzymology," Vol. XIX, ed by G.E. Perlman, and L. Lorand, Academic Press, New York, 1970, pp. 504—508.
12) *a*) A.J. Barrett and P.M. Starkey, *Biochem. J.*, **133**, 709 (1973); *b*) H. Rinderknecht and M.C. Geokas, *Biochim. Biophys. Acta*, **295**, 233 (1973).
13) *a*) K. Ohlsson, *Scand. J. Clin. Lab. Invest.*, **28**, 219 (1971); *b*) K. Katayama and T. Fujita, *Biochim. Biophys. Acta*, **336**, 165 (1974); *c*) *Idem, ibid.*, **336**, 178 (1974); *d*) *Idem, ibid.*, **336**, 191 (1974).
14) *a*) M. Di Rosa and D.A. Willoughby, *J. Pharm. Pharmacol.*, **23**, 297 (1971); *b*) M. Di Rosa, *ibid.*, **24**, 89 (1972).

kininase during first 1.5 hr and the marked suppression after that was good agreed with their finding.

On the other hand, Parratt and West showed that the egg white edema formation was suppressed by the substances which possess potent anti-histamine and anti-serotonin properties.[15] Rosa and Sorrentino also showed that the edema induced by egg white was quite unaffected by pre-treating the rats with cellulose sulfate, which caused the plasma kininogen I depletion as the result of plasma prekallikrein activation,[16] while the edema induced by carrageenin was suppressed by this treatment.[17] These findings obviously suggested that main mediators in egg white edema were histamine and serotonin and the kinin system plays no important role in this edema. The present enzyme suppressed this edema but the suppression was weak as compared with that of carrageenin edema by it. In addition, the dose dependency of Shimeji kininase on egg white edema was not observed. Therefore, the weak suppression of egg white edema by Shimeji kininase was considered not to be contradictory to their findings. The weak suppression of egg white edema by Shimeji kininase which has the potent kininase activity, however, deserves further investigation.

Intravenous injection of 500 U or more Shimeji kininase caused a slight but continuous fall of arterial blood pressure. The fall of blood pressure, however, was not thought to be due to the direct action of Shimeji kininase because the fall of blood pressure restored to the normal level within 10—15 min after the intravenous injection of 500 U of the enzyme although the enzyme still had the kininase activity in the vascular system even after this time. Namely, the restoration of the blood pressure was not the result of inactivation of Shimeji kininase itself. Thus, the fall of blood pressure would be taken place by other mechanism which was induced by the intravenous administration of Shimeji kininase. However, we have not the direct proof to explain this problem as yet.

Judging from these points, this enzyme was considered to be able to block the kinin action in the body for a certain period and its application to kallikrein-kinin system could be greatly expected.

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