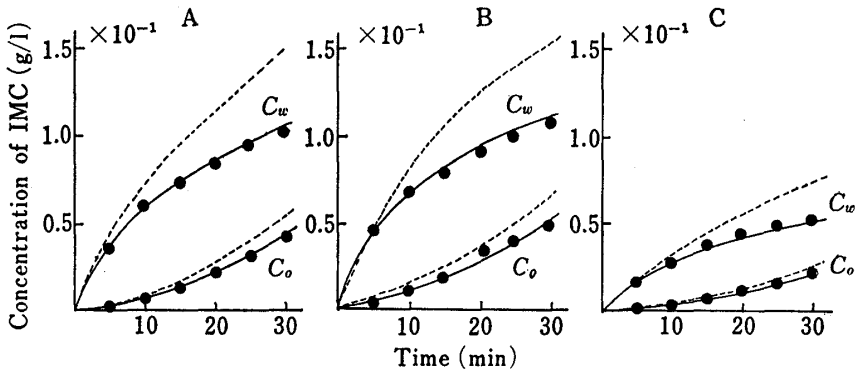

Fig. 4. Interfacial Transfer of IMC following Dissolution of IMC/PVP Coprecipitates at 37°C

A: IMC/PVP K-15 coprecipitate, B: IMC/PVP K-30 coprecipitate, C: IMC/PVP K-90 coprecipitate.
-●-: experimental data (each point is the mean of three determinations).
——: theoretical curve obtained from equations 3 and 4.
----: theoretical curve obtained from equations 2 and 3.

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Kinin-inactivating Enzyme from the Mushroom *Tricholoma conglobatum*. VI. Actions on Angiotensins I and II[1)]

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A potent kinin-inactivating enzyme from the mushroom *Tricholoma conglobatum*, Shimeji kininase, liberated angiotensin II and the dipeptide, H–His–Leu–OH, from angiotensin I. Thus, this enzyme was considered to have both kininase and angiotensin I converting activities like kininase II (angiotensin I converting enzyme, EC 3.4.15.1), which is widely distributed in mammals of various species.

On the other hand, this enzyme had angiotensinase activity in addition to angiotensin

I converting activity. However, the rate of angiotensin II hydrolysis was very slow as compared with that of kinin hydrolysis; the molar ratio of angiotensin II hydrolysis to bradykinin hydrolysis was about 1: 70.

Keywords——kinin-inactivating enzyme from mushroom; kinin; angiotensin; kininase; kallikrein-kinin system; angiotensin I converting activity

There have been many reports on the kallikrein-kinin system,[2] but the physiological and pathological significance of this system in the body is still not well understood. Thus, the authors have been working on the potent kinin-inactivating enzyme from the mushroom *Tricholoma conglobatum*, Shimeji kininase, in the hope that this would provide a tool for specifically blocking kinin action in the body,[1,3] as a means of evaluating the role and significance of this system. This enzyme cleaved –Gly^4–Phe^5– and –Pro^7–Phe^8– bonds in the bradykinin molecule and had the most potent kinin-inactivating activity of all known kininases obtained from plants.[3a] Its utility in studies of the kallikrein-kinin system as a blocker of kinin action has already been reported.[1,3]

In the present investigation, the authors studied the actions of this enzyme on angiotensins I and II.

Figure 1 shows the contractile responses of angiotensin I alone and the incubation mixture of Shimeji kininase plus angiotensin I assayed by the Magnus method using an isolated rat uterus. Shimeji kininase used was purified as described in our previous paper.[3a] It gave a single band in disc electrophoresis on 7% (w/v) polyacrylamide gel and had a specific activity of 480 kininase unit/E_{280}. One kininase unit is the amount of enzyme that can hydrolyze 1 μg bradykinin per min at 30°C and pH 7.4. As shown in Fig. 1, the contractile responses to the incubation mixture of Shimeji kininase and angiotensin I (196 ng) gradually increased with incubation time (1—33 min) (Fig. 1-B to -J) as compared with the response to 200 ng of angiotensin I only (Fig. 1-A). This increase can be explained by the conversion of angiotensin I to angiotensin II by Shimeji kininase, because the rat uterus is 10 to 40 times more sensitive to angiotensin II than to angiotensin I.[4] This view was confirmed by thin-layer chromatography, *i.e.*, when angiotensin I was incubated with Shimeji kininase for 10 min, angiotensin I gradually decreased and two fragments corresponding to angiotensin II and H–His–Leu–OH became detectable (Fig. 2-A). These two fragments increased in amount upon prolonged incubation (1 h) (Fig. 2-B). From these observations, it was concluded that Shimeji kininase had angiotensin I converting activity in addition to its potent kininase activity. Kininase II (angiotensin I converting enzyme, EC 3.4.15.1), which is widely distributed in mammals of various species, is well known to have both kininase and angiotensin I converting activities.[2] In the present investigation, it was revealed that Shimeji kininase also has both kininase and angiotensin I converting activities, and one of the positions of bradykinin cleaved by the action of Shimeji kininase (–Pro^7–Phe^8–) was identical with that cleaved by kininase II.[3a] In these respects, Shimeji kininase appears to have similar enzymatic properties to kininase II. However, these are quite different enzymes. Namely, kininase II is a kind of carboxydipeptidase, since it liberates the C-terminal dipeptide, H–Phe^8–Arg^9–OH, from bradykinin,[2] while Shimeji kininase is not a carboxydipeptidase type enzyme; this enzyme hydrolyzed the –Gly^4–Phe^5– bond in bradykinin more easily than the –Pro^7–Phe^8– bond.[3a]

On the other hand, as shown in Fig. 2-C, the amount of angiotensin II generated by the action of Shimeji kininase gradually decreased on prolonged incubation (4 h) and two unknown fragments were newly generated. This result indicated that Shimeji kininase also has angiotensinase activity in addition to angiotensin I converting activity, though its angiotensinase activity was weak as judged from the requirement for a long incubation period. Table I shows a comparison between the kininase and angiotensinase activities of this enzyme when assayed by the Magnus method using bradykinin and angiotensin II as substrates. As shown in this table, the angiotensinase activity was very weak as compared with the kininase activity; angio-

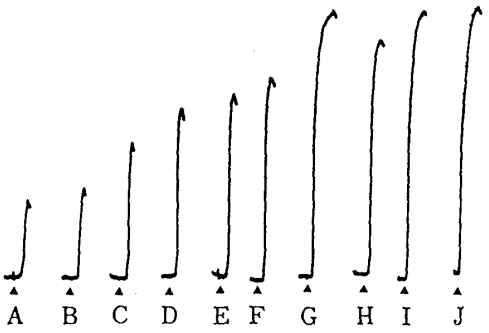

Fig. 1. Conversion of Angiotensin I to Angiotensin II by Shimeji Kininase

The Magnus assay was carried out as described in our previous paper.[3d] (A); angiotensin I, 200 ng. (B), (C), (D), (E), (F), (G), (H), (I) and (J); Ten μl of Shimeji kininase solution (4 kininase U/ml, in 0.01 M phosphate buffer, pH 7.4) was incubated with 0.5 ml of angiotensin I solution (10 μg/ml, in the same buffer) at 30°C, then 20 μl of this mixture (196 ng angiotensin I eq.) was added into the organ bath after 1, 3, 5, 7.5, 10, 15, 19, 25 and 33 min incubation.

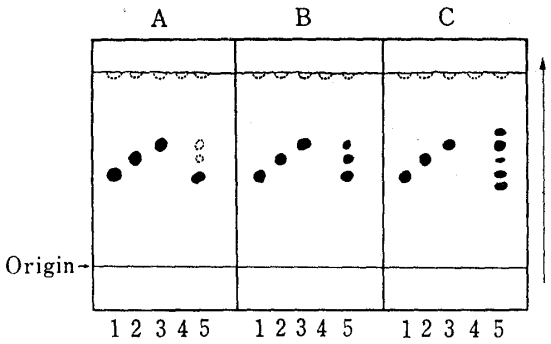

Fig. 2. Liberation of Angiotensin II and H–His–Leu–OH from Angiotensin I by Shimeji Kininase

1, 2 and 3; angiotensin I, angiotensin II and H–His–Leu–OH, respectively. To 50 μl of these peptide solutions (each 100 μg/ml), 50 μl of fluorescamine (F. Hoffmann-La Roche Co., Basel, Switzerland) solution (0.5 mg/ml in acetone) was added, and after 10 min aliquots were applied to silica gel 60 plates (5 × 20 cm, 0.25 mm, E. Merck, Japan). 4; 30 μl of Shimeji kininase solution (0.6 kininase U/ml) was treated with the same volume of fluorescamine solution and applied to the plates. 5; 30 μl of angiotensin I solution (1 mg/ml) was incubated with 30 μl of the enzyme solution (0.6 kininase U/ml) for 10 min (A), 1 (B) and 4 h (C). Then, they were treated with 60 μl of fluorescamine solution and aliquots were applied to the plates. The developing solvent was *n*-BuOH: AcOH: H_2O (4:1:2, v/v).

TABLE I. Kininase and Angiotensinase Activities of Shimeji Kininase

Substrate	Molecular weight	Hydrolyzed bradykinin or angiotensin II	
		μg/min/ml	nmol/min/ml
Bradykinin	1060	30.10	28.40(1)
Angiotensin II	1045	0.43	0.41(1/69)

Angiotensin II destruction by Shimeji kininase was determined by the Magnus method using an isolated rat uterus.[3d] For the assay of angiotensinase activity, 300 μl of angiotensin II solution (1 μg/ml in 0.01 M phosphate buffer, pH 8.0) was incubated with 300 μl of enzyme solutions of various concentrations at 30°C. After certain periods, 20 μl of this mixture (10 ng angiotensin II eq) was added to the organ bath and the remaining angiotensin II was determined. A standard curve for angiotensinase activity assay was prepared by the use of angiotensin II (2—10 ng). The rate of angiotensin II hydrolysis is shown in parenthesis based on bradykinin hydrolyzed, taken as 1.0.

tensin II hydrolysis was about 1/70 of that of bradykinin on a molar basis.

As mentioned above, it was revealed that Shimeji kininase had both angiotensin I converting and angiotensinase activities in addition to potent kininase activity. Such effects, however, are not serious obstacles to the practical application of Shimeji kininase as a blocker of kinin action in the mammalian body. Namely, application of Shimeji kininase would be useful to furnish one of evidences to reveal that kinin(s) would be involved. Whether the evidence obtained by the use of Shimeji kininase would be reasonable or not should be confirmed by other techniques. As reported previously, Shimeji kininase markedly suppressed anaphylactic shock in rats.[1] In this case, the authors carried out other detailed studies to determine whether the levels of kininogens, kinin, kininase and so on in plasma were changed or not in the shocked rats in addition to the application of Shimeji kininase, *i.e.*, the involvement of the kallikrein-kinin system in the anaphylactic shock was confirmed by additional experiments.

In any event, effects other than kinin destruction must be considered in relation to the kallikrein-kinin system when Shimeji kininase is practically applied as a blocker of kinin action in the body.

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