Angiotensin I Converting Enzyme and Kininase*

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A PEPTIDASE in plasma that converts angiotensin I to the octapeptide, angiotensin II, has been termed "converting enzyme".¹ A method of purification of this enzyme has been examined in our laboratory,² and the properties of the enzyme has been studied. It was suggested that the enzyme material had also a kininase activity.

The results of the efforts to separate a converting enzyme from a kininase activity are discussed in this paper.

MATERIALS AND METHODS

Substrates: Synthetic α -L-Asp¹-Ileu⁵-angiotensin I as the substrate for the estimation of converting enzyme activity, synthetic α -L-Asp¹-Ileu⁵-angiotensin II as the substrate for angiotensinase activity, synthetic bradykinin as the estimation of kininase activity were obtained from the Institute for Protein Research, Osaka University.

Assay of Angiotensin II and Bradykinin: Rat uterus which was much more sensitive to angiotensin II than to angiotensin I was used for the estimation of angiotensin II and bradykinin, the uterus was taken from a rat weighing approximately 200 g, injected previously with 1 mg of hexesterol per 1 kg of body weight. The rat uterus was suspended in a 5 ml isolated muscle chamber and one end attached by thread to a force-displacement transducer (Model SB-IT-H, Nihon Kohden). The uterus was kept at 1 gm tension and the contractions were recorded on Nihon Kohden polygraph. The bathing solution was kept at room temperature (20°C) and consisted of 0.9% of NaCl, 0.042% of KCl, 0.012% of

CaCl₂·2H₂O, 0.025% of dextrose, 0.05% of NaHCO₃ and 0.00053% of MgCl₂·6H₂O. Air was bubbled at all times through the bathing solution.

Assay of Converting Enzyme Activity: Angiotensin I solution (200 ng/ml of 0.9% NaCl solution in 1/100 M phosphate buffer, pH 7.2) was incubated with the enzyme material at 37°C for 15 minutes and reaction was stopped by placing the sample in a boiling water bath for 5 minutes. The amount of converted angiotensin II was assayed by means of the rat uterus assay and the enzyme activity was expressed as ng/mg/hr.

Assay of Kininase Activity: Synthetic bradykinin solution (200 ng/ml of 0.9% NaCl solution in 1/100 M phosphate buffer, pH 7.2) was incubated with enzyme material at 37°C for 15 minutes and reaction was stopped by placing the samples in boiling bath for 5 minutes. The amount of remaining bradykinin was assayed by means of the rat uterus assay.

Purification Procedures: Ammonium sulfate fractionation, DEAE-Sephadex A-50 column chromatography, DE-32 cellulose column chromatography, Sephadex G-200 gel filtration and Bio-Gel A 15 m gel filtration were employed as the purification procedures. In the first step the fraction between 1.6 M and 2.2 M of ammonium sulfate was used as the active fraction. DEAE-Sephadex A-50 column chromatography was performed using 0 to 0.5 M NaCl linear gradient elution in 1/100 M phosphate buffer, pH 7.2. DE-32 was also used in a same way as DEAE-Sephadex A-50. Sephadex G-200 and Bio-Gel A-15 m were used for gel filtration.

Electrophoresis with Horizontal Starch Block: The electrophoresis was conducted at pH 8.6 (veronal-veronal Na buffer $\mu = 0.05$) at 12 mA, 260 V (3.0 × 1.5 × 40.0 cm) for 16 hours.

Inhibition of Converting Enzyme and Kininase

Key Words: Angiotensin I
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Activities by Bradykinin Potentiating Peptide (BPP): BPP (Pyr-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ileu-Pro-Pro) was obtained from the Institute for Protein Research, Osaka University. For *in vitro* experiment, BPP was added in a different concentration to the reaction system for kininase and converting enzyme activities as mentioned above. The amount of destroyed bradykinin and the amount of formed angiotensin II were compared with control (without BPP) system. For *in vivo* experiment, the blood pressure in carotid artery of an anesthetized, pentrinium treated and vagotomized Wistar rat was recorded on kimo-graph. The responses of the blood pressure for angiotensin I were compared before and after the BPP injection (1 μ g, 10 μ g, 20 μ g and 50 μ g in 0.1 ml of saline) through femoral vein.

Preparation of the Enzyme from the Lung: The acetone powder of the lung were used as the lung enzyme material. The enzyme was extracted from the acetone powder with 1/100M phosphate buffer for several hours followed by centrifugation. The supernatant was fractionated with 70%

of ammonium sulfate and the precipitate was used for the enzyme material.

RESULTS

The results of purification of converting enzyme was shown in the Table I. In the step of DEAE-Sephadex A-50 column chromatography,

TABLE I PURIFICATION OF CONVERTING ENZYME

steps	total activity (ng/h)	specific activity (ng/mg/h)	recovery (%)	folds
plasma	1,479,600	137	100.0	1.0
(NH ₄) ₂ SO ₄ fractionation	525,245	349	35.4	2.6
DEAE-Sephadex A-50	163,440	908	11.0	6.6
Sephadex G-200	138,147	8,570	9.3	63.0

Fig.1. Patterns of converting enzyme and kininase activities.

the fraction showing angiotensinase activity was completely separated from converting enzyme fraction. The kininase activity was detected in the both of fractions. After the step of Sephadex G-200, the specific activity of converting enzyme was 8,570 ng/mg/hr showing 63 folds elevation of the original plasma. By using high voltage paper electrophoresis, it was demonstrated that this enzyme released a peptide His-Leu by cleaving Phe-His bond of angiotensin I.

The characters of the enzyme material were as follows; 1) release His-Leu from angiotensin I, 2) hydrolyse His-Leu, Leu-Gly, Leu-Gly-Gly and bradykinin, 3) do not hydrolyse angiotensin II, Cbz-Gly-Phe, Cbz-Gly-Arg, Leu- β -Naphthylamide, 4) optimum pH 7.2–7.5, 5) inhibited by 10^{-3} M EDTA.

The efforts to separate the converting enzyme activity from the kininase activity were performed in several ways. The results are shown in Fig.1. Chromatographic pattern on DE-32 cellulose showed no difference between converting enzyme activity and kininase activity. On the starch block electrophoresis these activities were demonstrated in the same position at the post-albumin position. The elution patterns on Bio-Gel A 15 m column showed also no difference between two enzyme activities.

As a possible method to differentiate these two enzyme activities, the bradykinin potentiating peptide B (BPP), which had been known to have the inhibitory activity of kininase, was examined if this BPP also had the inhibitory potency to converting enzyme activity.³ This BPP showed almost the same potency of inhibition on converting enzyme as on the kininase activity; that is, 10^5 pM of the BPP showed 95–100% inhibition on converting enzyme and 70–80% inhibition on kininase activity, 10^4 pM of the BPP showed 80% inhibition on converting enzyme and 50–60% inhibition on kininase activity. The BPP also inhibited converting enzyme activity *in vivo* in rat; 20 μ g of the peptide inhibited almost completely and 50 μ g of the peptide inhibited the enzyme activity completely.

The converting enzyme and the kininase in plasma and in the lung were compared using DEAE-Sephadex A-50 column chromatography and horizontal starch block electrophoresis. The converting enzyme in plasma and in the lung were eluted in the same molar or sodium chloride in linear gradient elution of NaCl on DEAE-Sephadex A-50 column chromatography, and moved in the same position on starch block elec-

kallikrein-kinin system

renin-angiotensin system

Fig.2.

trophoresis.

DISCUSSION

The characters of the converting enzyme had not been clear until very recently. In the purification step, we found that the enzyme preparation had both converting enzyme activity and kininase activity. We failed to separate both of the activities from each other. Yang *et al.*^{4,5} reported that the purified kininase II from plasma released His-Leu from angiotensin I and release Phe-Arg from bradykinin. They found also that this enzyme released dipeptide from Hip-His-Leu, Hip-Gly-Gly, Cbz-Pro-Leu-Gly and t-Boc-Phe(NH₂)-Phe-Gly, and they named this enzyme as "Carboxydiptidase or dipeptidyl carboxypeptidase". Elisseeva⁶ had found the unique enzyme "Carboxycathepsin" which released dipeptide from C-terminus of peptides, and he suggested that converting enzyme was carboxycathepsin itself. It is highly probable that these two enzymes are the same. So the converting enzyme produces a strong pressor peptide angiotensin II in the renin-angiotensin system and on the other hand acts as kininase II as it destroys bradykinin, a strong depressor peptide, in the kallikrein-kinin system.

It was demonstrated that the lung also had high activity of converting enzyme which showed the same character as in plasma. Ng *et al.*^{7,8} and Biron *et al.*^{9–11} reported that converting enzyme works mainly in the lung. If this is true, angiotensin II is characterized as a "circulating hormone" and bradykinin is characterized as "local

hormone". We may suspect that renin-angiotensin system has some relationship to kallikrein-kinin system in the metabolic pathway at the point of carboxydipeptidase through the pulmonary circulation (Fig.2).

SUMMARY

Angiotensin I converting enzyme was partially purified from rabbit plasma and from the lung. We failed in our efforts to separate converting enzyme from kininase activity using column chromatography, gel filtration, electrophoresis and inhibitor.

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CONCLUDING REMARKS:

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Questions were raised by many that if any organs other than lung were checked for the converting enzyme activity, and, if kidney is potent in that activity next to lung. It was answered that they are being investigated, and, so far, there was no difference between plasma and lung converting enzyme preparations, and the both resulted in only one peak activity in several chromatographies.

Although the investigation was carried out in vitro, several questions were raised as to the in vivo version, e.g., if any difference of converting enzyme activity was detected between pulmonary artery and vein, and if the pulmonary converting enzyme which the reporter has purified stays only inside the lung, or released to blood stream and act therein. These have not yet been investigated.

An another question concerning the in vivo conversion was that whether the bradykinin potentiating peptide (=kininase inhibitor) which inhibited converting enzyme in vitro, can also block converting enzyme in vivo and result in a reduction of the blood pressure in renovascular hypertension. This also has not been checked yet.

Since both the converting enzyme preparations, either from lung or plasma, were shown to have been contaminated with a dipeptidase activity, a possibility was questioned whether it was carboxypeptidase contaminated in the preparation that hydrolyzed histidyl-leucine bond and the incubation time was not long enough to proceed the reaction to hydrolyze prolyl-phenylalanine bond. But, it was answered that the incubation was done for 24 hours which is long enough, and, furthermore, data on the substrate specificity indicated that there was no possibility of carboxypeptidase contamination.

Since the reporter defined that kinin is a local hormone and angiotensin is a systemic hormone, it has raised several discussions on this. Finally, it seemed agreed upon by many that angiotensin is partly a systemic hormone, in that it acts in general arteriolar beds, and also partly a local hormone, in that it acts on local areas, such as thirst center in hypothalamus, kidney, uterine or salivary gland, as well.

To sum up the paper and discussion, the investigators have purified converting enzyme from either lung or plasma which also showed kininase II activity. Only contaminant so far detected was dipeptidase which hydrolyzed histidyl-leucine bond. These data were obtained exclusively in vitro experiment, and investigation in vivo is in progress.