Purification and Properties of Angiotensin I-Converting Enzyme from Rabbit Lung*

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ANGIOTENSIN I-converting enzyme which converts angiotensin I to angiotensin II by releasing the terminal dipeptide was first isolated from horse plasma. The enzyme is also thought to be capable of inactivating bradykinin and to be identical with kininase II. Purification of the enzyme has been attempted from plasma, kidney and lung of a few sorts of animals. Since this enzyme is thought to exist as a membrane-bound protein, there are some problems in solubilizing it from membrane fraction. Cheung and Cushman tried to extract the enzyme from acetone powders of rabbit lung. Soffer et al. and Oshima et al. attempted to solubilize the enzyme by using detergents. But, it is difficult to solubilize it in homogenous form with the above methods. So we investigated the effect of trypsin treatment on solubilization of the enzyme from rabbit lung.

Enzyme assays

Angiotensin I-converting enzyme assay was performed by the spectrophotometric method of Cushman and Cheung. One unit of the enzyme activity was defined as the amount of the enzyme which hydrolysed 1 μmol per min at 37°C under the conditions described by them. The other assay of the enzyme was performed using angiotensin I as substrate. The assays of angiotensinase and kininase activity were performed using angiotensin II and bradykinin as substrates. The biologically active peptides were assayed in the isolated rat uterus.

Purification of angiotensin I-converting enzyme from rabbit lung

Fresh rabbit lungs were chopped into small pieces and suspended in 20 mM potassium phosphate buffer, pH 7.8, containing 0.25 M sucrose. The suspension was homogenized in a Waring blender for 4 min and centrifuged for 20 min at 700 × g. The supernatant was filtered with two layers of gauze and adjusted to pH 5.2 with acetic acid and centrifuged for 20 min at 15000 × g. The pellet was suspended in 10 mM potassium phosphate buffer, pH 7.8 and adjusted to pH 7.8 with 1 M NaOH. The acid precipitated fraction was incubated with trypsin (1 mg/10 mg protein), containing 1 mM CaCl₂ for 120 min at 37°C and centrifuged for 90 min at 78000 × g. After the supernatant was concentrated with Amicon PM 10 filter, the sample was applied to a column of Sephadex G-200 (2.6 × 90.5 cm) which was equilibrated with 10 mM potassium phosphate buffer, pH 7.8. The enzyme activity was obtained as a single peak of molecular weight 300000. The active fraction was dialyzed for 48 h against 1 mM potassium phosphate buffer pH 6.8 and applied to a column of hydroxyapatite which was equilibrated with the same buffer. The enzyme absorbed on the column was eluted with a linear gradient of phosphate buffer increasing in molarity from 1 to 30 mM. The active fraction was applied to a column of DE 52-cellulose which was equilibrated with 5 mM potassium phosphate buffer, pH 7.8. The enzyme was eluted with a linear gradient of NaCl (0–0.5 M). The purification of this step was about 2000-fold with recovery of 9.8% of the enzyme activity present in the initial lung extract. The active fraction of DE 52-cellulose eluate showed only a single major protein band and two

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faint bands after 5% polyacrylamide disc gel electrophoresis. The properties of a major protein band and two faint bands were 94% and 6%, densitometrically. The gel was cut into 5 mm slices and incubated with 1 ml of 10 mM potassium phosphate buffer, pH 7.8 at 37°C for 24 h. The enzyme activity was detected in the same place as the major protein band. The final preparation obtained from disc gel fraction had a specific activity of 24.3 units/mg protein for Hipppuryl-His-Leu-OH and 0.182 μmol/min per mg protein for angiotensin I. Also, it was confirmed that the purified enzyme did not inactivate angiotensin II, but did inactivate bradykinin. The specific activity of the enzyme for inactivation of bradykinin was 2.6 μmol/min per mg protein. The purified enzyme was incubated with 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol for 2 h at 50°C. The molecular weight of the denatured enzyme was found to be 155000 by disc gel electrophoresis in the presence of sodium dodecyl sulfate.

The effect of trypsin treatment
The acid precipitated fraction was divided into five parts and each of them was incubated with trypsin (1 mg/10 mg protein) in the presence of 1 mM CaCl₂ for 1, 2, 3, 4 and 5 h at 37°C, respectively. After the reaction had been stopped with soybean trypsin inhibitor (1 mg/1 mg trypsin), each sample was centrifuged for 90 min at 78000 × g. Recovery of each activity as compared with the total enzyme activity of initial fraction was 40.4, 76.0, 57.7, 55.5 and 59.0% respectively. The enzyme activity obtained after trypsin treatment for 5 h could be divided into two components: (i) an enzyme of molecular weight 30000 and (ii) an enzyme of molecular weight 145000, by Sephadez G-200 gel filtration. In respect to the difference of molecular weight of enzyme calculated by gel filtration and by disc gel electrophoresis, there are two possibilities: (i) the enzyme fraction of molecular weight 300000 may contain the dimer of the enzyme, or (ii) the difference may be only due to the presence of carbohydrate, which was reported by Soffer et al. Km values of the enzyme of molecular weight 300000 and 145000 for Hipppuryl-His-Leu-OH were identical and 2.6 mM.

Discussion
Chairman: Kikuo Arakawa, M.D., Fukuoka Univ.

CHAIRMAN: A new preparation and the characterization of converting enzyme from rabbit lung was presented. Is there any questions or comments?

Dr. K. KANEKO (Yokohama City Univ.): Do you have any clinical data which supports that the lung converting enzyme is really contributing to the regulation of the systemic blood pressure?

Drs. K. NISHIMURA & T. KOKUBU (Ehime Univ.): There is no definite and direct clinical data, but hypotension or shock during the introduction of artificial heart-lung-machine in cardiac surgery is well known and that might well be the case, even though we do not have any data of converting enzyme lebel. But we do have same data in animals, i.e.; in those animals in which pulmonary diseases were introduced or SQ 20881 was administered, a reduction of blood pressure together with converting enzyme activity were seen.

Dr. T. TAKEDA (Tokyo Univ.): It is well known that SQ 20881 inhibits converting enzyme and causes a decrease of angiotensin II formation, thereby also decreasing feedback inhibition on renin release. It results in hyperreninemia with a reduction of blood pressure. By the same token, is there also similar hyperreninemia in your pulmonary disease in animals?

Dr. T. KOKUBU: We have not determined it.

Dr. K. YAMAMOTO (Osaka City Univ.): Do the both preparations of molecular weight of 300,000 and 145,000 have the same specific activity on the substrate, either angiotensin I or bradykinin?

Dr. K. NISHIMURA: Specific activity was checked using only the preparation of 300,000.

CHAIRMAN: Is not there a possibility that your preparation of molecular weight of 145,000 still a dimer in conjunction with the report by Nakajima and Fitz who estimated the molecular weight as 70,000? How about trying enzymes other than trypsin too?

Dr. K. NISHIMURA & T. KOKUBU: It is very much possible, but, the use of trypsin has some advantages over the use of detergent which split both hydrophilic and hydrophobic portion, together with the membrane. Trypsin is supposed to split only hydrophilic portion. The minimum active size thus obtained happened to be the molecular weight of 145,000. Further degradation so far tested by trypsin seems to destroy the active center of the enzyme.

CHAIRMAN’S COMMENTS: The authors presented a novel method of preparing converting enzyme from rabbit lung. They used trypsin
in place of detergent, in an attempt to split the
membrane bound enzyme at the minimum size
(molecular weight: 145,000) with the active
center intact.
After several steps of procedure, the highly
purified preparation was shown to be homo-
geneous in the disc-electrophoresis. Whether this
is the minimum subunit, or still can be subfrac-
tionated is remained for further investigation.