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ANGIOTENSIN I-CONVERTING ENZYME OF THE KIDNEY CORTEX*

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SUMMARY

We purified the angiotensin I-converting enzyme (peptidyldipeptide hydrolase; kininase II, EC 3.4.15.1) from homogenized hog kidney cortex. For the sake of comparison, we also purified the enzyme from homogenized human lung, human kidney and hog plasma. The microsomal fraction of the homogenized swine kidney cortex contained eight times more enzyme than the corresponding fraction from homogenized swine lung. The hog kidney enzyme had a molecular weight of 195 000. It contained about 8% neutral sugars. No subunits of the converting enzyme were observed. The enzyme liberated angiotensin II from angiotensin I and inactivated bradykinin. It also hydrolyzed the fluorescent substrate 1-dimethylaminonaphtalene-5-sulfonylglycylglycylglycine and three other substrates that were measured in the ultraviolet spectrophotometer. The ratios of rates of cleavage of dipeptides from the C-terminal end of three optically active substrates were similar for the hog plasma, kidney, and lung enzymes, but different from the human enzymes. Antibody to hog converting enzymes cross-reacted with hog lung and plasma enzymes, but not with converting enzyme of human kidney. All the enzymes tested were completely inhibited by 10^{-4} M of the peptide inhibitor SQ 20881. The absence of Cl^- from the buffer induced conformational changes in the structure of purified enzyme in the ultraviolet spectrophotometer.

INTRODUCTION

The importance of renin as a locally acting hormone of the kidney has been emphasized [1] often. Renin, however, releases only angiotensin I that is enzymatically converted to the biologically more active angiotensin II by the removal of C-terminal histidyl-leucine. This angiotensin I-converting enzyme was first detected in blood [2]. Later the lung was found to be a rich source of this enzyme [3]. In 1966 we found an

Abbreviations: DNS-, 1-dimethylaminonaphtalene-5-sulfonyl-; Bz-Gly-Gly-Gly, hippurylglycylglycine; Bz-Gly-His-Leu, hippurylhistidylleucine; BOC-Phe-Phe-Gly, tert-Boc-*p*-nitrophenylalanylphenylalanylglycine.

* Some of these results were summarized at a meeting of the Council for High Blood Pressure Research of the American Heart Association held in Cleveland, Ohio, October, 1973.

enzyme in kidney cortex that inactivated bradykinin by cleaving the C-terminal dipeptide terminus of the nonapeptide. The activity was concentrated in the microsomal fraction of the homogenized kidney cortex [4, 5]. This kininase (named kininase II or peptidyldipeptide hydrolase) was found to be identical with the angiotensin I-converting enzyme [6–8]. We purified it first from plasma [6, 7] and subsequently from lung [8, 9].

Because the presence of large amounts of converting enzyme in the kidney may indicate an important function, we undertook the study of the renal angiotensin I-converting enzyme and compared its properties with those of the purified lung enzyme.

MATERIALS AND METHODS

Converting enzyme was purified from swine kidney cortex in a cold room. Fresh swine kidneys were obtained from a slaughterhouse. The blood from the kidneys was removed by perfusion with cold saline. 200 g of kidney cortex were chopped and suspended in 1600 ml of 0.25 M sucrose buffered with 0.05 M Tris, pH 7.4, and were homogenized for 4 min in a Waring blender. The homogenate was centrifuged for 30 min at 5000 $\times$ *g* in a Sorvall refrigerated centrifuge. The precipitate was discarded and the supernatant was centrifuged again for 2 h at 50 000 $\times$ *g* in a Beckman L-2 65 ultracentrifuge. The precipitate was suspended in 1600 ml of 0.26% deoxycholate in 0.25 M sucrose buffered with 0.05 M Tris, pH 7.4, with a glass homogenizer at 37 °C. The extract was centrifuged again at 50 000 $\times$ *g* for 2 h in the ultracentrifuge.

After the precipitate had been removed, supernatant was concentrated to about one-tenth of the original volume with an Amicon XM-100 membrane filter under N_2 pressure. The concentrate was filtered through a Sephadex G-200 column (10 cm $\times$ 90 cm) equilibrated with 0.05 M Tris, pH 7.4 containing 0.1 M NaCl. The rate of gel filtration was 20 ml/h and fractions of 18 ml were collected. The fractions in the second active peak (tubes 80 to 140, see Results) were pooled and concentrated on an Amicon filter. The concentrated active fraction was dialyzed against two changes of 5 l of 5 mM Tris, pH 7.4, overnight. The dialyzate was divided into two parts, and each was adsorbed on a column (6 cm $\times$ 40 cm) of DEAE-Sephadex A-50 equilibrated with the same buffer. The activity was eluted with 0.05 M Tris and a linear gradient of NaCl concentration increasing from 0 to 0.3 M. The flow rate was 43 ml/h, and 14-ml fractions were collected. The active fractions were again combined and concentrated on the membrane filter. The concentrate was dialyzed overnight against 5 l of 1 mM phosphate buffer, of pH 6.8 containing 5 mM NaCl. The concentrate was applied on a column (2.5 cm $\times$ 35 cm) of hydroxyapatite equilibrated with the same buffer. The proteins were eluted from the column with a linear gradient of phosphate buffer (pH 6.8) containing 5 mM NaCl and increasing in phosphate molarity from 1 mM to 0.05 M. The active fractions were gel filtered on a column (2.5 cm $\times$ 87 cm) of Sephadex G-200 equilibrated with 0.05 M Tris, pH 7.4, and 0.1 M NaCl; 3.7-ml fractions were collected at the rate of 12 ml/h.

Rabbits received 3.4 ml of an equal mixture of converting enzyme (0.33 mg/ml in saline) and complete Freund's adjuvant (Difco) that had been emulsified with a Servall Omnimixer. Each animal was injected intramuscularly with 0.1 ml of the emulsified antigen in each thigh and subcutaneously 1.0 ml in the neck. The same

schedule was repeated 14 days later. A blood sample was taken 2 weeks later, 3 days after the intradermal injection of 1 ml of converting enzyme in saline (0.33 mg/ml). The harvest bleedings were begun 1 week later.

The source of antigen was converting enzyme purified from swine kidney cortex obtained through the steps shown in Table I, including DEAE-Sephadex A-50 column chromatography.

The antibody was purified on DEAE-cellulose column (2.5 cm × 40 cm) according to Sober and Peterson [10] and by gel filtration on Sephadex-G 200 column (2.5 cm × 110 cm) according to Flodin and Killander [11].

Agar-gel plates were made with 1.5% agar in 0.05 M Tris, pH 7.4, containing 0.1 M NaCl and 0.1 mM *p*-chloromercuriphenyl sulfonate. 5 μl of antisera and enzyme solutions were used on the plate. After 3 days of diffusion at room temperature, the excess protein was removed by immersion in saline, containing 1 mM phosphate buffer, pH 8.0. Inorganic salts were then removed with distilled water. After drying, the gel was stained with 0.1% Coomassie brilliant blue in 50% methanol for 10 min. The excess dye was washed off with 50% methanol.

Disc gel electrophoresis, preparative disc gel electrophoresis, iso-electrofocusing, and protein determination and molecular weight determinations by disc gel electrophoresis in presence of sodium dodecylsulfate have been described in the previous publications [8, 9].

The activity of the enzyme was established by chemical and biologic techniques. In the ultraviolet spectrophotometric experiments, hippurylglycylglycine (Bz-Gly-Gly-Gly), hippurylhistidylleucine (Bz-Gly-His-Leu), and *tert*-Boc-*p*-nitrophenylalanylphenylalanylglycine (BOC-Phe-Phe-Gly) were the substrates. The details of the assays have been reported elsewhere [7, 9]. They are based on measuring the hydrolysis of C-terminal dipeptides cleaved by converting enzyme from the substrates at a wavelength of 254 or 310 nm. One unit is that amount of the enzyme that cleaves 1 μmole of substrate in 1 min.

Qualitatively, the enzyme activity was also shown in thin-layer chromatography using 1-dimethylaminonaphthalene-5-sulfonyl-glycylglycylglycine (DNS-Gly-Gly-Gly) as fluorescent substrate [8]. After 2 h of incubation in 0.06 ml of reaction mixture 0.5 μg of converting enzyme cleaved the fluorescent product DNS-Gly that migrated faster than the substrate and was localized under the long wavelength of the ultraviolet light.

The inactivation of bradykinin by converting enzyme was followed on the isolated rat uterus [9]. The same bio-assay was used for determining the conversion of angiotensin I to angiotensin II, since this organ is more sensitive to the octa than to the decapeptide [12]. The concentration of bradykinin in the incubation mixture was 0.1 μg/ml; that of angiotensin was 0.5 μg/ml.

The inhibition of converting enzyme by antiserum was determined by spectrophotometric experiments and by bio-assay. In spectrophotometric experiments, 0.05 mg/ml of antibody was added to 0.7 μg of enzyme and incubated for 30 min before adding the substrate. In biologic experiments either rabbit antiserum that was heated for 30 min at 61 °C was used (0.05 ml for each incubation mixture) or 0.05 ml of purified antibody.

For comparison, converting enzyme was also purified from human kidney as described above, from hog lung [7], and from hog plasma. Citrated swine plasma (50

ml) was dialyzed overnight in the cold room against 4 l of 1 mM phosphate buffer, pH 6.8, containing 5 mM NaCl. The dialysate was centrifuged at 5000 × *g* for 15 min in a Sorvall refrigerated centrifuge. The supernatant was applied to a column (2.5 cm × 43 cm) of hydroxyapatite, buffered with the same buffer. Proteins were eluted from the column with linear gradient of phosphate buffer increasing in molarity from 1 mM to 0.05 M (pH 6.8) containing 1 mM NaCl. The flow rate was 38 ml/h and 14 ml per tube was collected. The converting enzyme activity was eluted with 5 mM phosphate. Swine plasma converting enzyme was purified 40-fold. 1 mg protein of the preparation cleaved 0.05 μmole Bz-Gly-Gly-Gly per min.

Because human tissues were obtained from cadavers, the activities cannot be compared with those of the hog enzyme extracted from organs removed immediately after death. The best preparation of human renal enzyme cleaved 11.6 μmoles of Bz-Gly-Gly-Gly per min per mg protein; the human lung enzyme hydrolyzed 16.7 μmoles of the substrate. The nonapeptide inhibitor of the converting enzyme SQ 20881 was used after 20 min of preincubation with the enzyme [7, 8, 13–18].

RESULTS

Purification

The purification steps for the converting enzyme from hog kidney are summarized in Table I. The calculations are based, however, on data from experiments using

TABLE I

PURIFICATION OF CONVERTING ENZYME FROM HOG KIDNEY CORTEX

Purification step	Volume (ml)	Units/ml	Total units	Protein (mg/ml)	Spec. act.* (units/mg)	Yield (%)	Purification
Deoxycholate extract of microsomal fraction	1150	1.144	1315.6	8.81	0.17	100	1
Sephadex G-200 gel filtration	1100	0.47	526.5	1.79	0.27	39.9	1.6
DEAE-Sephadex A-50 column chromatography	20	12.9	257.6	1.95	6.6	19.6	39.3
Hydroxyapatite column chromatography	4.9	11.2	102.9	0.76	27.8	7.9	165.7
Sephadex G-200 gel filtration	33	2.3	76.6	0.09	27	5.8	160.8

* Substrate Bz-Gly-Gly-Gly.

the deoxycholate extract of the microsomal fraction of kidney cortex homogenate as the starting material. When no deoxycholate was added after the first separation of the microsomal fraction, (see Methods), the supernatant had 10 times less activity than it had in the presence of added deoxycholate. Homogenized hog kidney cortex contained about 50% more enzyme in the final supernatant than hog lung, but the deoxycholate extract of the renal fraction precipitate was 8 times more active per g of

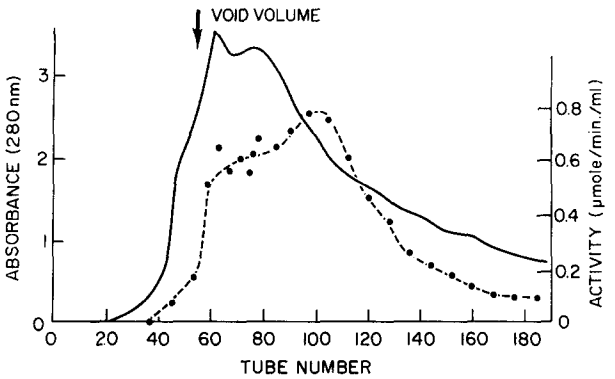

Fig. 1. Sephadex G-200 gel filtration of the deoxycholate extract of microsomal fraction from swine kidney cortex. Abscissa: Tube number. Ordinates: Absorbance was measured at 280 nm and activity was experessed as μmoles of Bz-Gly-Gly-Gly hydrolyzed per min. Solid line: protein concentration; ●—●, enzyme activity.

tissue than the corresponding fraction from the lung. During the first Sephadex G-200 gel filtration, most of the protein was eluted near the void volume with a broad tailing. The enzyme activity separated into two peaks. The first appeared with the main protein peak, and the second enzymic activity appeared in the shoulder region of the protein. The fractions containing the main enzyme activity were purified further (Fig. 1). Because of the pore size of the membrane filter used, we assume that most of the material with a molecular weight below 100 000 had already been removed from the extract. Chromatography on DEAE-Sephadex (Fig. 2) and hydroxyapatite columns (Fig. 3) and the subsequent Sephadex G-200 gel filtration yielded 3 mg of enzyme protein per 200 g of tissue.

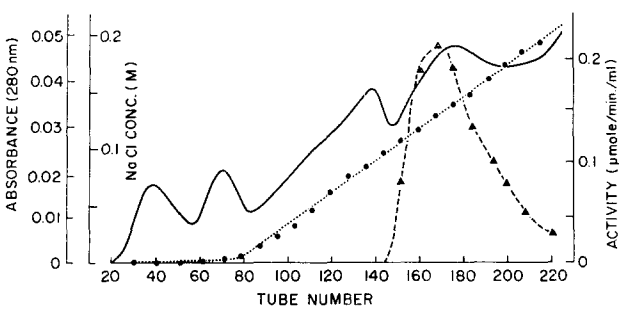

Fig. 2. DEAE-Sephadex A-50 column chromatography of the renal converting enzyme. See Legend to Fig. 1. ●- - -●, NaCl concentration; ▲- - -▲, enzyme activity.

Disc gel electrophoresis of converting enzyme revealed only a single major protein band, but an additional faint band was seen due to an estimate 5–10% impurity. This impurity was removed in a preparative polyacrylamide electrophoresis [8].

Molecular weight

The molecular weight of converting enzyme was determined in polyacrylamide-gel electrophoresis in presence of sodium dodecyl sulfate. Plotting the log of

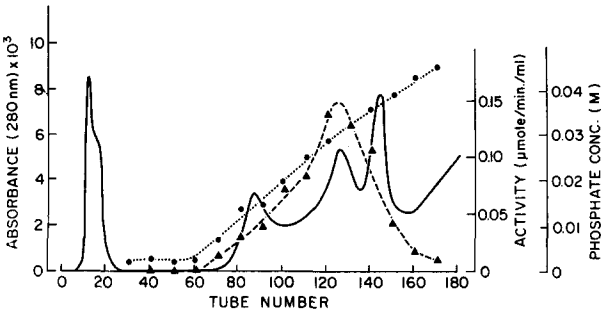

Fig. 3. Hydroxyapatite column chromatography of converting enzyme. See Legend to Fig. 1. ●- - -●, phosphate concentration; ▲- - -▲, enzyme activity.

the molecular weight of standard proteins against the ratios of distance of migration yielded a straight line. Using this plot, we estimated the enzyme to have a molecular weight of 195 000. This value was, within experimental error, identical with the molecular weight of the lung angiotensin I-converting enzyme [9]. No additional protein bands were detected when converting enzyme was incubated for 1 h at 60 °C in 5% mercaptoethanol, 1% sodium dodecyl sulfate and 8 M urea. This experiment indicated that the enzyme contained no dissociable subunits.

Isoelectric focusing

The p*I* value of the enzyme was established by isoelectric focusing. After 3 days of electrofocusing in the ampholine buffers at an initial 700 V and 9 mA, the p*I* was 5.2.

Substrates

The purified converting enzyme of the kidney hydrolyzed all the substrates of the lung and plasma enzyme tested. The relative rates of hydrolysis of Bz-Gly-Gly-Gly, Bz-Gly-His-Leu and BOC-Phe-Phe-Gly by purified kidney and lung enzymes were close to those obtained with partially purified plasma (Table II).

The ratios of the relative rates of hydrolysis of the substrates by human lung and kidney converting enzyme (Table II) were similar, but different from those of the hog enzyme.

In thin-layer chromatography experiments, the converting enzyme cleaved DNS-Gly-Gly-Gly to DNS-Gly and Gly-Gly.

TABLE II

RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES BY CONVERTING ENZYME OF LUNG, KIDNEY, AND PLASMA

Substrate	Source of enzyme:	Kidney cortex		Lung		Plasma (Porcine)
		Human	Porcine	Human	Porcine	
Bz-Gly-Gly-Gly		1	1	1	1	1
Bz-Gly-His-Leu		1.17	0.39	1.04	0.33	0.48
BOC-Phe-Phe-Gly		0.4	0.21	0.63	0.21	0.22

Like purified plasma or lung enzyme, the purified renal converting enzyme inactivated bradykinin and converted angiotensin I to angiotensin II. Under our conditions of the bio-assay the time for inactivation of 50% of bradykinin present by 5 μg/ml of hog kidney enzyme was 3.6 min and that of angiotensin I conversion was 19.9 min. The corresponding figures for the same concentration of purified hog lung converting enzyme were 2.2 min with bradykinin and 16.1 min with angiotensin I substrate.

The inhibitor SQ 20881 at 10^{-4} M concentration inhibited the enzymic cleavage of all the substrates used in spectrophotometry, in bio-assay and in thin-layer chromatography.

Conformational changes

The converting enzyme needs Cl^- and a divalent cation for cleaving substrates in vitro, although the Cl^- requirement is not absolute. The effect of Cl^- on the conformation of the enzyme was studied by dialyzing the converting enzyme against 2 × 1 l of Cl^--free Tris acetate buffer, pH 7.4, for 2 days at 4 °C. The difference spectra of the protein solution were recorded in the ultraviolet spectrophotometer containing a Cl^--free preparation in the reference cell compartment of the instrument. The Cl^--free enzyme in the sample cell compartment showed a shift at 37 °C to lower absorption at the wavelength between 260 and 290 nm (Fig. 4). When solid NaCl was added to the sample cell to bring up the concentration to 0.2 M, the blue shift disappeared and the absorption increased over the control throughout the ultraviolet range.

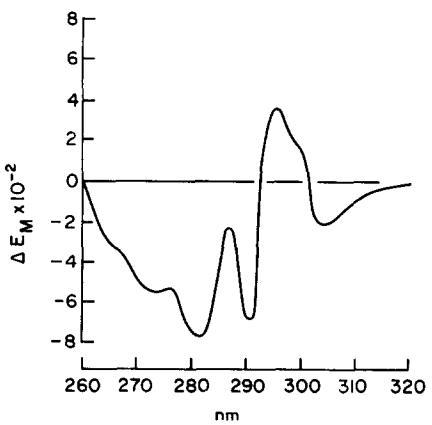

Fig. 4. Difference spectrum of Cl^--free enzyme preparation. Blue shift in absence of Cl^-.

The Cl^--free enzyme preparation cleaved Bz-Gly-Gly-Gly when NaCl was added at 4 °C immediately before starting the reaction (Fig. 5-2). A low rate of hydrolysis was observed when the substrate was added to the Cl^--free enzyme solution at 4 °C before starting the recording of the reaction (Fig. 5-3), indicating that the substrate could partially protect the enzyme against conformational changes. When the enzyme was incubated for 30 min at 37 °C prior to adding the substrate, no hy-

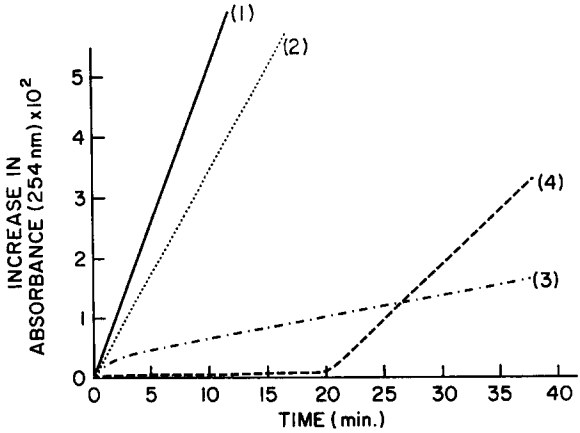

Fig. 5. Effect of Cl^- on the rate of cleavage of Bz-Gly-Gly-Gly. 1. Activity of renal converting enzyme in 0.05 Tris–acetate containing 0.1 M NaCl. 2. Enzyme preparation dialyzed free of Cl^-, but the buffer added contained NaCl, final concentration 0.1 M. 3. Buffer contained no Cl^-, no preincubation at 37 °C. 4. Buffer contained no Cl^-, but enzyme was preincubated at 37 °C for 30 min before adding substrate. After 20 min, NaCl was added. Abscissa: time in min. Ordinate: increase in absorbance at 254 nm.

drolysis was observed. After 20 min solid NaCl was added in the cuvette of the recording spectrophotometer, and the activity partially returned (Fig. 5-4).

Carbohydrate content

After disc electrophoresis, the gel was stained with fuchsin-sulfine [16]. A pink band developed that corresponded to the location of the enzyme in the gel. This indicated that the converting enzyme is a carbohydrate containing protein. This was further confirmed by neutral sugar analysis done according to Dubois et al. [17]. The glucose equivalent content was 8% per weight.

Immunologic studies

Because rabbit serum contains both converting enzyme and an inhibitor of converting enzyme [7], we found it difficult to measure the inhibition of hog kidney converting enzyme by untreated antiserum. Thus, in the spectrophotometric experiments, we used purified antibody. This antibody preparation contained neither converting enzyme nor an inhibitor of it.

Table III summarizes the spectrophotometric studies. The purified antibody

TABLE III

INHIBITION (%) OF CONVERTING ENZYME BY PURIFIED RABBIT ANTIBODY TO HOG KIDNEY ENZYME

Substrate	Source of enzyme: Hog kidney	Hog lung	Hog plasma	Human kidney
BOC-Phe-Phe-Gly	75	78	87	15
Angiotensin I	50	38	35	0
Bradykinin	46	42	48	0

inhibited only the hydrolysis of BOC-Phe-Phe-Gly, but not that of Bz-Gly-Gly-Gly substrate significantly. Obviously this was due to the structure of the substrates, indicating that the antigenic site of the enzyme differed from the hydrolytic site. Hence, the hydrolysis of substrate of bulkier structure was inhibited by the antibody. Thus, the purified antibody inhibited the hydrolysis of Bz-Gly-Gly-Gly and Bz-Gly-His-Leu by the kidney enzyme only 14 and 23%, while the hydrolysis of BOC-Phe-Phe-Gly was blocked 75%.

The antibody blocked the cleavage of BOC-Phe-Phe-Gly by hog kidney, lung and plasma enzymes similarly, but it was ineffective against human kidney converting enzyme, thereby showing species specificity. The cleavages of both angiotensin I and bradykinin by the three types of enzyme preparations were inhibited 50, 38, and 35% resp. 46, 42, and 48% respectively by the purified antibody as shown in bio-assay. Rabbit serum taken before immunizing the animal and heated to 61 °C for 30 min inhibited the inactivation of bradykinin by 37 and 28% by the purified hog kidney and lung enzymes.

Immunodiffusion studies supported the results of the enzyme inhibition experiments. Purified swine kidney and lung converting enzyme and partially purified converting enzyme from swine plasma produced a single precipitin band with antiserum against swine kidney converting enzyme. Purified human kidney converting enzyme, however, did not react with the antiserum.

DISCUSSION

Our data indicates that the angiotensin I-converting enzyme is highly concentrated in the renal cortex. A particulate fraction of the hog kidney cortex that sedimented at a high speed had about eight times more extractable enzyme than the corresponding fraction obtained from hog lung. The homogenous protein has a molecular weight of about 190 000 and contains neutral sugars. The renal enzyme closely resembles the lung enzyme. Homogeneous enzyme preparations from either organ, or partially purified from plasma, converted angiotensin I to angiotensin II, inactivated bradykinin, and cleaved C-terminal dipeptides from three optically active substrates at a similar rate. The molecular weight of the lung enzyme is close to that of the renal enzyme. Antibody induced by injecting purified renal enzyme as antigen to rabbits cross-reacted with the lung and plasma enzyme of the hog. This was shown by the formation of a precipitin band on agar plate and by the inhibition of substrate hydrolysis. The latter depended, however, on the structure of the substrate. While the hydrolysis of Bz-Gly-Gly-Gly was not significantly inhibited by the purified antibody, those of BOC-Phe-Phe-Gly, bradykinin, or angiotensin I were. Thus, the cleavage of the bulkier substrates was inhibited while that of the smaller (Bz-Gly-Gly-Gly) was not. This indicates that the antigenic and hydrolytic sites of the enzyme protein are different. The antibody attached to the antigenic site partially blocks the hydrolytic center sterically.

For the sake of comparison in preliminary studies, the enzyme was purified from human tissues as well. However, antibody to hog kidney enzyme did not cross-react with the human enzyme. The relative rates of hydrolysis of three substrates by human kidney and lung enzymes were similar to each other but different from those obtained with the hog enzyme (Table II).

Although the antigenic groups of the various hog enzymes are quite similar, the structure of the hog kidney and lung converting enzymes cannot be identical. Under the combined effects of heating, urea, and sodium dodecyl sulfate the lung enzyme released subunits of 70 000 mol. wt, while the kidney enzyme seemed to consist of a single peptide chain. In addition in the homogenized lung, a dimer and a form of the converting enzyme of even higher molecular weight were observed [9].

An enzyme in the microsomal fraction of the hog kidney cortex that cleaved C-terminal dipeptides from bradykinin was described by Yang and Erdös in 1966 first [4, 5], but the identity of this kininase II with the converting enzyme was not shown until 1970 [6]. Others also observed the kidney to be a rich source of a converting enzyme or kininase II and purified the renal enzyme, although the details of the purification were not revealed [18]. The introduction of the use of shorter peptide substrates of converting enzymes in chemical assays [6, 19–21] expedited the research. It was also shown that during purification the ratio of the rate of cleavage of Bz-Gly-Gly-Gly [22] or Bz-Gly-His-Leu [13] to that of angiotensin I stayed constant. Thus, all of these are the actions of a single enzyme protein.

The fact that the hydrolysis of bradykinin depended less on the presence of Cl^- in the media than that of angiotensin I puzzled many investigators. It was postulated that kininase II, which inactivates the hypotensive peptide differs from the enzyme that liberates angiotensin II from angiotensin I [23]. Using the purified lung enzyme it was later shown that the structure of the substrate determines the Cl^- dependence of the enzyme [24]. In addition, although in the absence of Cl^- the soluble enzyme is ineffective against angiotensin I, the insoluble Sepharose-converting enzyme complex still hydrolyzes the peptide at about one-fourth of the normal rate. In absence of Cl^-, the inactivation of bradykinin by the soluble enzyme is slower but still proceeds [9, 24]. Finally, Cl^- did not accelerate the cleavage of a pentapeptide substrate of converting enzyme and chloride was described as an "allosteric" modifier of the enzyme [15].

In our studies, the ultraviolet spectra of the purified enzyme indicated that the renal converting enzyme had a different configuration in the presence than it did in the absence of Cl^-. Obviously bradykinin can be attached to the enzyme and be hydrolyzed in both configurations, while Bz-Gly-Gly-Gly is cleaved or angiotensin I is converted only in the configuration where the tryptophan and the tyrosine residues are more accessible, as indicated by the red shift in the ultraviolet spectrum. Adding substrate to the enzyme immediately before starting the reaction partially protected the converting enzyme against unfavorable changes caused by Cl^--free buffer medium.

Converting enzyme was present in all the blood samples and tissues we investigated [24]. Initially we did not detect kininase II (or converting enzyme) in rabbit plasma [25]. This lack of activity was caused either by inactivation of the enzyme due to freezing of the plasma samples tested, or by the absence of enzyme due to genetic variations. In addition, just as guinea pig plasma [7], rabbit plasma contains an inhibitor of converting enzyme.

Converting enzyme located in the lung [7, 8, 26–28], especially in the vascular endothelium may affect the metabolism of circulating hypo- and hypertensive peptides, e.g. bradykinin and angiotensin I. Although the kidney can be a richer source of converting enzyme than the lung, the action of the renal converting enzyme is not evident when angiotensin I is infused in the kidney. This is so because only a small portion of the peptide appears as angiotensin II in the effluent [26, 29]. Thus, possibly

in contrast to the lung, the renal converting enzyme has a local, intrarenal function [30] while the pulmonary enzyme would influence the systemic circulation. It follows that renin would be an important local hormone of the kidney by affecting the function of this organ and by releasing of other active materials [31].

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