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## High Molecular Weight Renin in the Mouse Kidney

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This study was conducted to determine whether or not high molecular weight renin is present in the mouse kidney. The molecular weight of renin in the cytosol fraction of mouse kidney cortex was approximately 40000 as determined by gel filtration. Renin in this fraction was partially converted into high molecular form (54000) in the presence of sodium tetrathionate or *N*-ethylmaleimide, which are sulfhydryl group blockers. This high molecular weight renin was converted into regular size renin by acidification and trypsin treatment. In contrast, the molecular weight of renin in the granules was 40000, regardless of the absence or presence of sulfhydryl group blockers. Furthermore, neither change in molecular weight nor change in activity was observed after acidification. These results indicate that regular size renin is stored in the granules and may be converted into high molecular weight renin when it is released from the granules and reacts with some substance in the cytosol fraction of renal cortical tissue.

**Keywords**—mouse kidney cortex; discontinuous sucrose-density gradient centrifugation; cytosol fraction; renin granules; high molecular weight renin; regular size renin; acidification; trypsin

### Introduction

In recent years, there have been a number of reports on high molecular weight renin in the kidneys and plasma in various species.<sup>1-4)</sup> However, the physiological significance and molecular properties of the high molecular weight renin remain unclear.

In mice, the submaxillary gland is one of the richest sources of renin, together with the kidneys, and contains the only active renin with a molecular weight of 40000.<sup>5,6)</sup> On the other hand, morphological studies have shown that mouse kidney renin is stored in the granules of juxtaglomerular cells,<sup>7,8)</sup> but little is known regarding the characteristics of renin in the kidney. Recently, we have isolated renin granules from the mouse kidney cortex.<sup>9)</sup>

The aim of the present work was to investigate the storage form of renin in the granules and to clarify whether or not high molecular weight renin is present in the mouse kidney.

### Materials and Methods

**Experimental Animals and Preparation of Tissue Homogenate**—Male albino mice of ddY strain weighing 25–30 g were used. The animals were decapitated without anesthesia. Both kidneys were immediately removed and placed in cold physiological saline. The cortex was sectioned into thin slices with a blade at low temperature and rinsed thoroughly with cold physiological saline to remove as much blood as possible.

**Preparation of the Cytosol Fraction and Renin Granules of the Kidney Cortex**—The cytosol fraction and renin granules fraction were prepared by discontinuous sucrose-density gradient centrifugation as previously described by us.<sup>9,10)</sup> The soluble renin from the granules was prepared according to the method of Funakawa *et al.*<sup>11)</sup> These fractions were subjected to gel filtration.

**Determination of Molecular Weight of Renin**—The molecular weight of renin was estimated by gel filtration. One ml of sample was applied to a Sephadex G-100 column (1.6 × 90 cm, Pharmacia) equilibrated with 40 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl and 3 mM NaN<sub>3</sub> as an antimicrobial agent at 4°C. The flow rate was 8.8 ml/h and 1 ml fractions were collected. The void volume (V<sub>0</sub>) of the column was estimated by using blue dextran (Pharmacia). Bovine serum albumin (BSA) (M.W. 67000), ovalbumin (M.W. 45000),  $\alpha$ -chymotrypsinogen A (M.W. 25000) and cytochrome C (M.W. 12900) were used as molecular weight standards. All these standard proteins were purchased from Sigma Chemical Co.

**Acidification**—Aliquots of the cytosol fraction and of the soluble renin from the granules were dialyzed against 50 mM glycine-HCl buffer (pH 3.0) containing 0.1 M NaCl at 4°C for 20 h and then neutralized by dialysis against 40 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at 4°C for 20 h.

**Activation with Trypsin**—Samples were incubated with 1 mg/ml of trypsin (Bovine Type III, Sigma) in 40 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at 37°C for 15 min and then the reaction was stopped by the addition of soybean trypsin inhibitor (10 mg/ml).

**Modification of Sulfhydryl Groups**—To examine the effect of sulfhydryl group blockers on the conversion of regular size renin (M.W. 37000–43000) to high molecular weight renin, sodium tetrathionate (5 mM) and *N*-ethylmaleimide (10 mM) were used to oxidize sulfhydryl groups. Dithiothreitol (50 mM) was used for the purpose of investigating the conversion from high molecular weight renin to regular size renin.

**Renin Assay**—Since angiotensinases and converting enzyme are present in the kidney tissue, renin activity was determined by incubating the sample under conditions such that these enzymes were inactive. The incubation mixture consisted of: a) samples, 0.05 ml; b) renin substrate dissolved in 25 mM sodium phosphate buffer (pH 7.0) containing 25 mM disodium ethylenediaminetetraacetic acid, 0.5 ml; c) 5% diisopropyl-fluorophosphate, 20  $\mu$ l; d) 6.6% 8-hydroxyquinoline sulfate, 10  $\mu$ l; e) 10% dimercaprol, 3  $\mu$ l. Incubation was carried out at 37°C for 30 min, and the reaction was stopped by the addition of 0.1 ml of 1 N HCl. Angiotensin I generated in the incubation mixture was determined by radioimmunoassay by using the CEA-IRE-SORIN kit. Renin activity was expressed as ng of angiotensin I (AI)/ml/h.

**Preparation of Renin Substrate**—Rat renin substrate was prepared according to the procedure described previously.<sup>9)</sup> In brief, the rats were bilaterally nephrectomized 24 h before bleeding, and blood samples were collected from the aortic cannula into syringes moistened with 15% disodium ethylenediaminetetraacetic acid or heparin solution. The blood samples were immediately cooled in ice and centrifuged at 10000 rpm for 20 min at 4°C. Renin substrate was prepared from plasma according to the procedure described previously.<sup>12)</sup> Renin and angiotensinase activities were absent in this substrate solution. Renin substrate yielded 12.5  $\mu$ g of AI per ml, when incubated with an excess of rat renin.

## Results

### Molecular Weight of Renin in the Cytosol Fraction

The elution profiles of renin in the cytosol fraction of the mouse kidney cortex from a Sephadex G-100 column are shown in Fig. 1. Renin activity in the cytosol fraction showed a single peak with the molecular weight of  $40000 \pm 1000$ . To examine the effect of acidification on renin activity, the cytosol fraction was acidified before application to the column. Significant changes in molecular weight and renin activity were not observed (Fig. 1).

It has been shown that blockers of sulfhydryl groups are required for the conversion of regular size renin to high molecular weight renin.<sup>13)</sup> In the present experiment, when sodium tetrathionate (5 mM) was added to the cytosol fraction and incubated at 0°C for 30 min before gel filtration, renin was eluted in two peaks with molecular weights of  $54000 \pm 2000$  and  $40000 \pm 1000$  (Fig. 2A). Similarly, when *N*-ethylmaleimide (10 mM) was substituted for sodium tetrathionate, three peaks of enzyme activity were observed in the elution profiles, corresponding to molecular weights of  $54000 \pm 2000$ ,  $45000 \pm 1000$ , and  $40000 \pm 1000$  (Fig. 2B). These elution patterns of renin activity were unchanged in the presence of increasing amounts (50 mM) of sulfhydryl blockers.

Dithiothreitol (50 mM) was added to the cytosol fraction pretreated with sodium tetrathionate. The mixture was incubated at 37°C

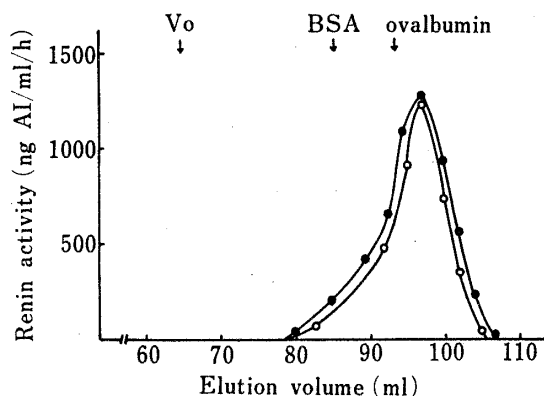


Fig. 1. Elution Profile of Renin in the Cytosol Fraction on Gel Filtration

A portion (0.5 ml) of the sample was applied to a Sephadex G-100 column. Renin activity was measured before (●) and after (○) acidification. Elution positions of molecular weight standards are indicated by arrows; void volume (Vo), bovine serum albumin (BSA, M.W. 67000), ovalbumin (M.W. 45000). For experimental details, see the text.

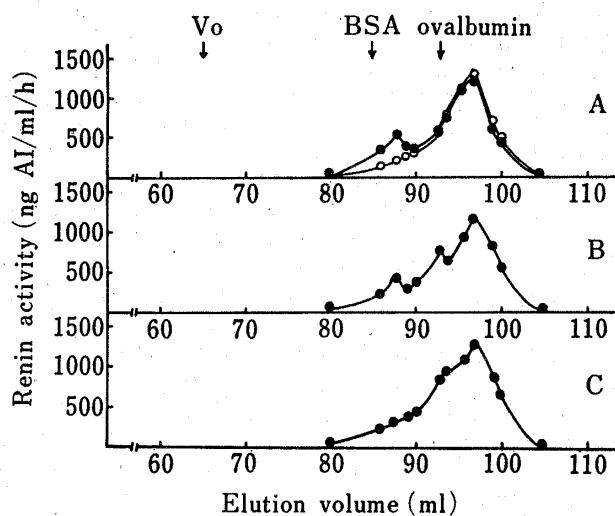


Fig. 2. Gel Filtration Profile of Renin in the Cytosol Fraction after Pretreatment with Sulfhydryl Group Blockers

Open circles show the elution profile of renin in the cytosol fraction. A) The cytosol fraction was incubated with 5 mM sodium tetrathionate at 0°C for 30 min before gel filtration. B) The cytosol fraction was incubated with 10 mM *N*-ethylmaleimide at 0°C for 30 min before gel filtration. C) The cytosol fraction was incubated with 5 mM sodium tetrathionate at 0°C for 30 min, then the mixture was further incubated with 50 mM dithiothreitol at 37°C for 15 min. The experimental conditions and symbols are the same as those in Fig. 1.

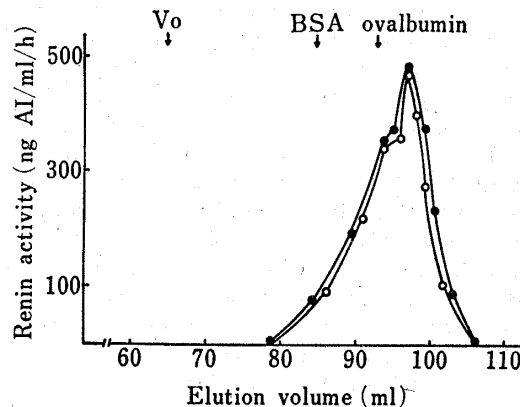


Fig. 3. Elution Profile of Renin in the Granules on Gel Filtration

One ml of the sample was applied to a Sephadex G-100 column. Renin activity was measured before (●) and after (○) acidification. The other symbols are the same as those in Fig. 1.

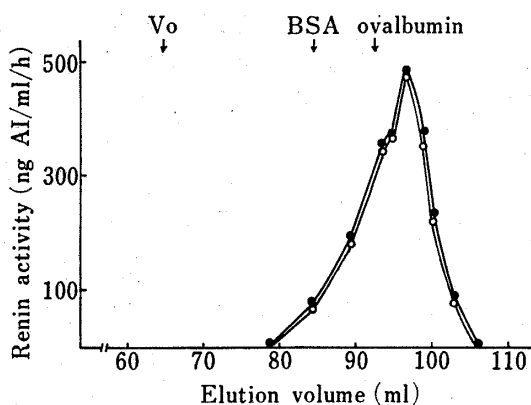


Fig. 4. Elution Profile of Renin in the Granules Pretreated with Sodium Tetrathionate.

Closed circles show the elution profile of renin in the granules. Open circles show the elution profile of renin in the granules pretreated with sodium tetrathionate. The other symbols are the same as those in Fig. 1.

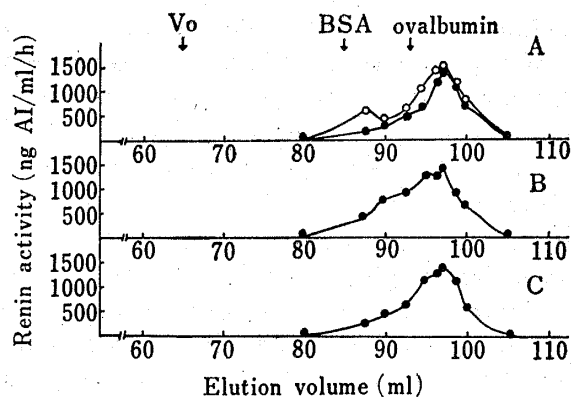


Fig. 5. Effects of Acidification and Trypsin on the Elution Profile of Renin in the Cytosol Fraction Pretreated with Sodium Tetrathionate

Open circles show the elution profile of renin in the cytosol fraction pretreated with 5 mM sodium tetrathionate. A) Renin activity was measured after acidification. B) Renin activity was measured after incubation with trypsin. C) The cytosol fraction was acidified before incubation with sodium tetrathionate. The other symbols are the same as those in Fig. 1.

for 15 min, and then applied to a Sephadex G-100 column. Renin was eluted in a uniform peak with a molecular weight of  $40000 \pm 1000$  (Fig. 2C). These findings indicate that the conversion between high molecular weight renin and regular size renin in the cytosol fraction of the mouse kidney cortex is reversible.

### Molecular Weight of Renin in the Granules

The molecular weight of renin in the granules was approximately  $40000 \pm 1000$  as shown in Fig. 3. This renin did not change its molecular weight and activity after acidification (Fig. 3). Furthermore, even if sodium tetrathionate or *N*-ethylmaleimide was added to renin in the granules before gel filtration, no renin activity could be detected at a position corresponding to the high molecular weight renin (Fig. 4).

### Effects of Acidification and Trypsin on Gel Filtration of Renin in the Cytosol Fraction pretreated with Sodium Tetrathionate

While renin in the cytosol fraction pretreated with sodium tetrathionate was eluted as two peaks with molecular weights of  $54000 \pm 2000$  and  $40000 \pm 1000$ , renin was eluted entirely as a regular size form when this fraction was acidified and subjected to gel filtration (Fig. 5A). Furthermore, when high molecular weight renin was incubated with trypsin and subjected to gel filtration, the activity of high molecular weight renin disappeared (Fig. 5B).

The conversion of regular size renin into high molecular weight renin could not be observed in cytosol fraction which had been acidified before the addition of sodium tetrathionate (Fig. 5C).

### Discussion

This paper is the first report on a high molecular weight renin in the mouse kidney. In the present study, only active renin of regular size was observed in the cytosol fraction. However, when renin in the cytosol fraction was incubated with sodium tetrathionate or *N*-ethylmaleimide, it was partially converted into high molecular weight form. These results indicate that regular size renin can be converted into high molecular weight renin in the mouse kidney, as has been reported to occur in other species.<sup>14-18</sup> Poulsen and Nielsen<sup>19</sup> reported that high molecular weight renin could not be observed in the mouse kidney extract in the presence of various protease inhibitors. This discrepancy might be due to the difference of experimental conditions: 1) our preparations were obtained by discontinuous sucrose-density gradient centrifugation, 2) we added a sulfhydryl group blocker to the sample and then incubated it at 0°C for 30 min before application to the column and 3) the strain of experimental animals in the present study was different from that used in their work.

Some investigators suggested that sulfhydryl groups are very important in the interconversion between high molecular weight renin and regular size renin. Inagami *et al.*<sup>13</sup> reported the exclusive existence of high molecular weight renin in the hog kidney extract in the presence of sodium tetrathionate, and observed the same results in the rat kidney extract treated with *N*-ethylmaleimide. Funakawa *et al.*<sup>11</sup> demonstrated that regular size renin in the dog kidney was completely converted into high molecular weight form under similar conditions. However, in the cytosol fraction of the mouse kidney, the interconversion between the two forms of renin was not complete in spite of the addition of large amounts of sulfhydryl group blockers. Thus, high molecular weight renin in the mouse kidney may be converted more easily into regular size renin than those in the hog, rat or dog kidney.

As described in this paper, renin in the cytosol fraction was eluted in two peaks after the addition of sodium tetrathionate but in three peaks after the addition of *N*-ethylmaleimide. This discrepancy might be explained by the different reactivity of these reagents, because *N*-ethylmaleimide can react with sulfhydryl and amino groups.<sup>20</sup> Recently Kawamura *et al.*<sup>21</sup> reported that regular size renin of dog kidney was converted into high molecular weight renin after cold storage without protease inhibitors. Therefore, there is a possibility that the two forms of renin in the mouse kidney can be interconverted *via* mediators other than sulfhydryl groups.

No significant change in the activity of regular size renin in the cytosol fraction was

observed after acidification. Furthermore, when renin in the cytosol fraction was acidified before the addition of sodium tetrathionate, an irreversible conversion of high molecular weight renin into regular size form was observed. It has been reported<sup>15)</sup> that acidified cytosol fraction of the dog kidney cortex did not form high molecular weight renin in the presence of sodium tetrathionate. These findings suggest that the renin-binding ability of some substance in the cytosol fraction is lost upon acidification.

There are many reports on the activation of inactive renin in human plasma by serine proteases such as trypsin and kallikrein.<sup>22-25)</sup> In the present study, we examined the effect of trypsin on the molecular conversion of renin. The addition of trypsin to high molecular weight renin resulted in conversion into regular size renin without apparent intermediate forms, suggesting the involvement of endogenous protease in the conversion of high molecular weight renin. Trypsin is well known to hydrolyze only ester or peptide bonds in which the carboxyl moiety is that of lysine or arginine. It is assumed that the specific covalent bond for trypsin is located near the binding site for renin in the renin-binding substance of the cytosol fraction. Further experiments seem necessary to clarify the conversion mechanism by trypsin.

On the other hand, renin in the granules was of regular size regardless of the absence or presence of sulfhydryl group blockers. Further, renin in the granules was not activated appreciably by acidification. These results indicate that the renin-binding substance is absent in the granules and that renin in the granules exists as active renin.

In conclusion, regular size renin is stored in renin granules of the mouse kidney and can be converted into high molecular weight form when it is released from the granules and binds some substance in the cytosol fraction of renal cortical tissue.

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