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LOW MOLECULAR WEIGHT RENIN AS A STORAGE FORM IN RENIN GRANULES OF THE DOG

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SUMMARY: The molecular weight of renin extracted from isolated renin granules of the dog was estimated by gel filtration, using tetradecapeptide as substrate, and was approximately 43,000 daltons. Neither big renin nor big big renin was demonstrable. On the other hand, crude extract of kidney cortex showed angiotensin I generating enzymes other than 43,000 dalton form of renin, whose molecular weight were over 100,000 and around 70,000 daltons. They seemed nonspecific proteases, since they hydrolyzed tetradecapeptide but not plasma angiotensinogen. Therefore renin is stored in the renin granules as a low molecular weight form.

As for studies on the molecular weight of renin (E.C.3.4.99.19), two representative forms have been documented in the kidney and plasma; one is a low molecular weight form ranging from 37,000 to 43,000 daltons, while the other is a high molecular weight form ranging from 55,000 to 70,000 daltons (1-5).

Previously we reported that 43,000 dalton form of renin was contained in the crude extract of dog kidney, and it was converted into the 60,000 dalton form of renin by adding sodium tetrathionate to oxidize sulfhydryls in the extract. On the other hand, isolated renin granules contained the 43,000 dalton form of renin and the 60,000 dalton form was not demonstrable even by the addition of sodium tetrathionate (6,7). Thus we suggested that 43,000 dalton form of renin in the renin granules can be converted into the 60,000 dalton form if it has a chance to meet with some constituent of kidney extract, presumably cytoplasm.

Recently, however, several investigators reported a further high molecular weight form of renin in the kidney (8,9).

The evidence of our previous work (6,7) was restricted within the molecular conversion of renin between the 43,000 dalton form and the 60,000

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dalton form. Therefore, in this study we examined whether such renin-like enzyme with extremely high molecular weight is stored in renin granules in the dog kidney, using *TDP as the renin substrate because it is more sensitive to renin-like enzymes than plasma angiotensinogen (5).

MATERIALS AND METHODS

1) Crude extract of kidney cortex and renin in the granules of the dog
Crude extract of kidney cortex and renin in the granules were prepared as described before (7,10). These procedures are shown in Fig. I.

2) Enzyme inhibitors
In order to prevent destruction of renin, various inhibitors were added to 0.3 M sucrose solution before homogenization. Metalloproteases were inhibited with EDTA (5mM), serine proteases were inhibited with phenylmethanesulfonyl fluoride (2mM) and diisopropyl fluorophosphate (0.2mM), and in some cases sodium tetrathionate (5mM) was used to oxidize sulfhydryls.

3) Estimation of molecular weight of renin
The molecular weight of renin was estimated as described before (7).

4) Renin assay
Renin activity was measured by radioimmunoassay of **AI generated by incubation at 37°C. The incubation mixture consisted of 0.05 ml of sample, 0.05 ml of TDP (2,000 ng AI/ml), 0.4 ml of buffer containing 10 mM EDTA, 3.2 mM 2,3-dimercapto-1-propanol and 1.6 mM 8-hydroxyquinoline sulfate. In order to examine renin activity at various pH, 50 mM glycine/HCl buffer containing 0.1 M NaCl(pH 3.0), 100 mM phosphate buffer (pH 5.0 and 7.0) were used. Neomycin sulfate was added to the reaction mixture to give a concentration of 0.2 mg/ml. The reaction was stopped by cooling after incubation for 20 min or more. Semipurified dog plasma angiotensinogen (11) was also used instead of TDP to compare renin activity.

5) Acidification
Acidification was performed as described before (6).

6) Measurement of acid protease activity
Each fraction after gel filtration was incubated at 45°C with bovine hemoglobin for measurement of acid protease (13). Incubation mixture consisted of 0.25 ml of sample, 0.25 ml of bovine hemoglobin solution (8% w/v) and 0.5 ml of 50 mM glycine/HCl buffer containing 0.1 M NaCl (pH 3.0). Incubation was stopped with 5 ml of trichloracetic acid (3% w/v) after 2h. After centrifugation at 3,000 rpm, acid soluble peptides in the supernatant were determined by the method of Lowry et al.(14).

RESULT

Fig. 2-A and B show the elution profile of activity of renin in the granules from Sephadex G-100. The enzyme activity as the result of incubation with TDP was approximately 10 times that with angiotensinogen, and in both cases the activity was more prominent at pH 5.0 than at pH 7.0. The

*TDP: tetradecapeptide

**AI: angiotensin I

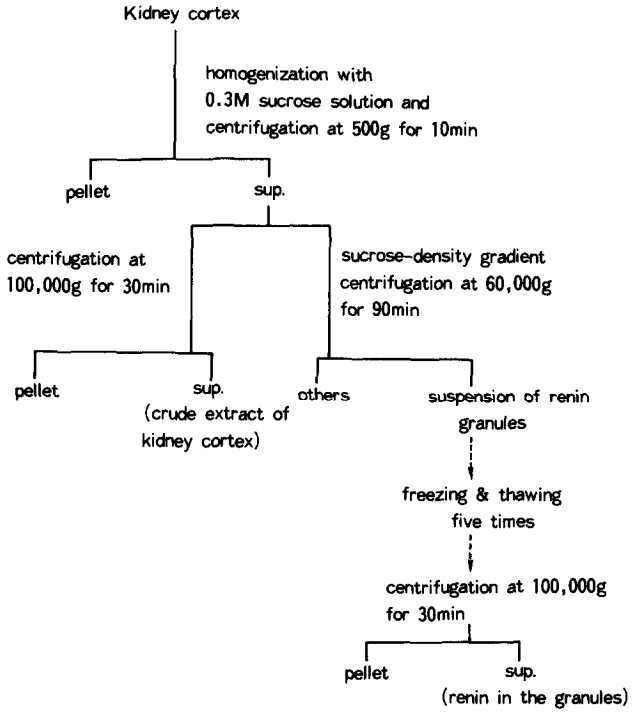

Fig. 1. Preparation of renin in the granules and crude extract of kidney cortex.

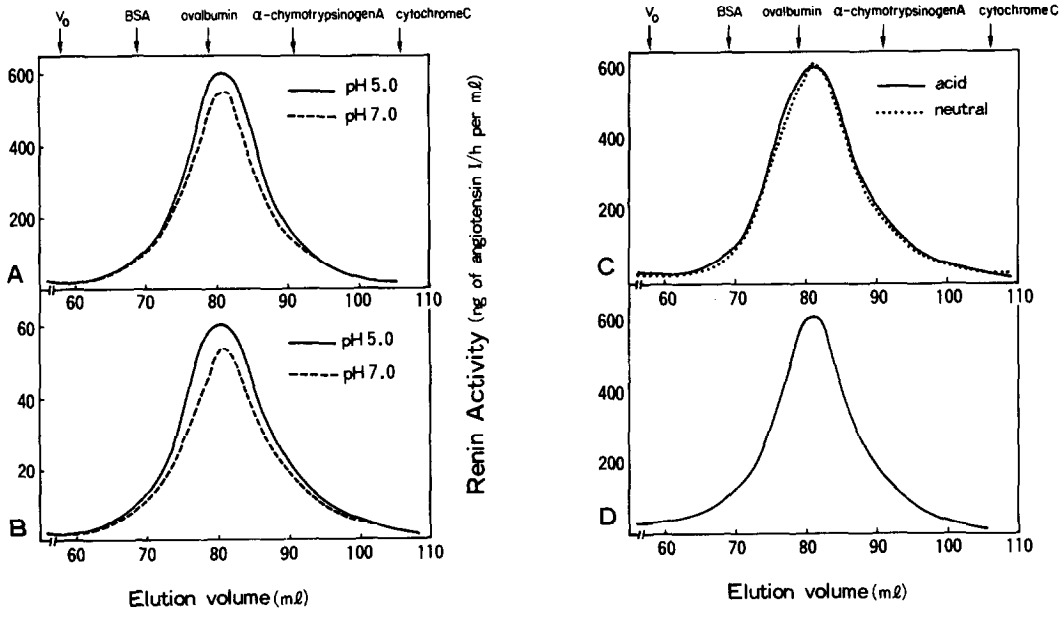

Fig. 2. Gel filtration profiles of renin in the granules. Renin activity was measured after incubation with different substrate at pH 5.0 (solid line) or pH 7.0 (broken line).
A) Renin activity was measured after incubation with TDP for 20 min.

elution patterns showed a single peak at 43,000 daltons. In an attempt to detect very low renin activity, incubation time was prolonged to 24 h for each fraction more than 50,000 daltons, but we could not find any high molecular weight form of renin in all four cases. To examine whether masked enzyme activity appears by acidification, each fraction of renin in the granules from gel filtration was acidified. No enzyme activity appeared from void volume to 60,000 daltons (Fig. 2-C). Sodium tetrathionate might lead to conversion of the 43,000 dalton form of renin into a higher molecular weight form. This was examined by adding 5 mM sodium tetrathionate to renin in the granules 30 min prior to application to the column. Again no enzyme activity appeared from void volume to 60,000 daltons (Fig. 2-D). By the technique used in this study for measuring renin activity, at least 200 pg AI/ml/h of renin is theoretically detectable (in this respect 24 h of incubation with TDP was needed). The peak activity after gel filtration of renin in the granules is, as shown in Fig. 2-A, 600 ng AI/ml at pH 5.0. Accordingly, if an activity more than 1/3,000 of the peak activity is present in any other fraction at a higher molecular weight range, such activity should be detected. Nevertheless, renin activity was not observed other than at 43,000 daltons.

On the other hand, the elution profiles of the crude extract of kidney cortex showed three peaks of enzyme activity when each fraction was incubated with TDP; void volume fraction (over 100,000 daltons), approximately 70,000 and 43,000 daltons (Fig. 3-A). When TDP was replaced with angiotensinogen, the peak of activity was observed at 43,000 daltons alone and other peaks were not detected (Fig. 3-B) even incubation time was prolonged to 24 h. It was assumed that enzyme at void volume and 70,000 daltons

B) Renin activity was measured after incubation with plasma angiotensinogen for 1 h.
C) Renin activity was measured after incubation with TDP for 20 min after acidification (———) or neutral dialysis (.....) of each sample.
D) Sodium tetrathionate was added to renin in the granules before gel filtration and then renin activity was measured after incubation with TDP for 20 min.

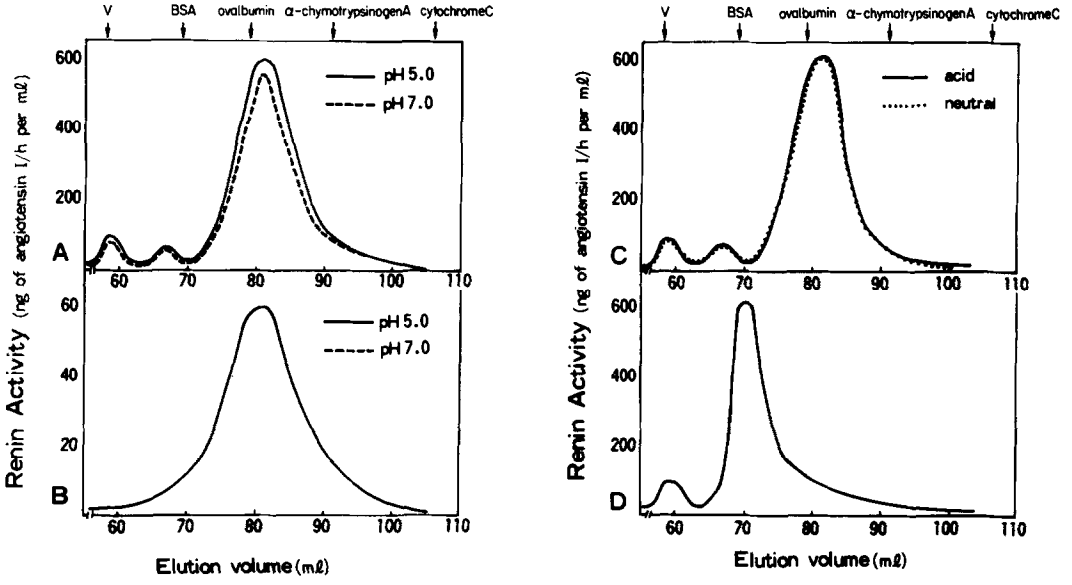

Fig. 3. Gel filtration profiles of crude extract of kidney cortex. Renin activity was measured after incubation with different substrate at pH 5.0 (solid line) or pH 7.0 (broken line).
A) Renin activity was measured after incubation with TDP for 20 min.
B) Renin activity was measured after incubation with plasma angiotensinogen for 1 h.
C) Renin activity was measured after acidification (———) or neutral dialysis (·····).
D) Sodium tetrathionate was added to crude extract of kidney cortex before gel filtration and then renin activity was measured after incubation with TDP for 20 min.

hydrolyze not angiotensinogen but TDP. Acidification of the crude extract of kidney cortex resulted in no change in elution profiles, indicating that renin-like activities at void volume and 70,000 daltons are not converted into lower molecular weight form by acidification (Fig. 3-C). When sodium tetrathionate was added to crude extract of kidney cortex before gel filtration, the activity at 43,000 daltons completely disappeared and the peak appeared at 60,000 daltons. The small peak at 70,000 daltons was incorporated into the 60,000 daltons activities, and the void volume fraction remained unchanged (Fig. 3-D).

In order to compare activity of acid protease with that of renin or renin-like enzymes, every examined sample was incubated at pH 3.0 with bovine hemoglobin, TDP and angiotensinogen. Renin in the granules did not

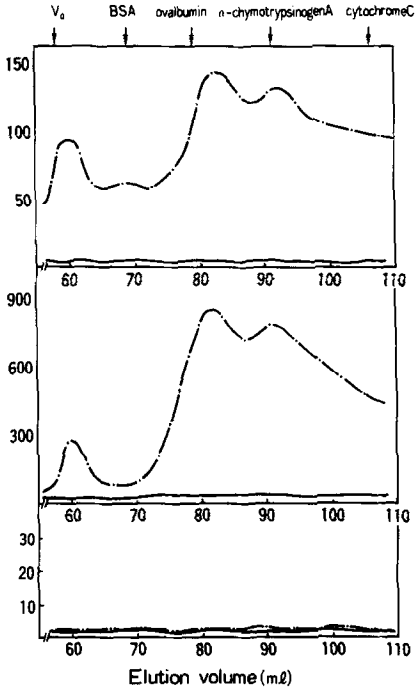

Fig. 4. Enzymatic activity of renin in the granules (———) and crude extract of kidney cortex (—·—·—) at pH 3.0.
A) Acid protease activity
B) Angiotensin I generating activity with TDP
C) Angiotensin I generating activity with plasma angiotensinogen

hydrolyze any substrate, therefore this sample did not include acid protease and true renin did not seem to act at pH 3.0. On the contrary, the crude extract of kidney cortex had acid protease activities at void volume fraction, 40,000 daltons and 25,000 daltons. Quite similar patterns were observed in AI generating activity by incubation at pH 3.0 with TDP, but not angiotensinogen, showing that acid protease in the crude extract of kidney cortex also hydrolyzes TDP but not angiotensinogen to liberate AI.

DISCUSSION

A high molecular weight form of renin ranging from 55,000 to 70,000 daltons is generally termed "big renin". Another renin-like enzyme having a molecular weight over 100,000 daltons was named "big big renin". It has been generally considered that they might be storage forms of renin. However, our present study clearly indicated that renin granules of the dog contained neither big renin nor big big renin.

On the other hand, in crude extract of kidney cortex we demonstrated high molecular renin-like activities both in void volume fraction and at 70,000 daltons. These enzymes generate AI from TDP but not from angiotensinogen, and did not change their molecular weight by acidification. These findings lead to the assumption that such enzymes are likely to be nonspecific proteases. In fact, gel filtration profiles of the kidney cortical extract proved that the elution pattern of acid protease activity closely resembles that of AI generating activity with TDP, indicating that acid protease also hydrolyzes TDP.

Funakawa et al. and Kawamura et al. demonstrated that the 60,000 dalton form of renin was produced by the action of renin binding substance in the cytosol fraction of crude extract of dog kidney cortex (7,12) on the 43,000 dalton form of renin. Thus, big renin was proved to be not a storage form. We also support their evidence with our finding described herein.

Nielsen et al. also reported that the molecular weight of renin in the mouse submaxillary gland is exclusively 40,000 and therefore the storage form of renin is unlikely to be a zymogen (15). The determination of molecular weight of the storage form of renin will be more required in various species.

ACKNOWLEDGMENT

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