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## Molecular Characterization of Renin in Plasma and Kidney of Sodium-Restricted Rats

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The present study was carried out to determine the molecular weight of renin and to investigate the presence of an acid-activatable form of renin (inactive renin) in the plasma and kidney of rats after dietary sodium restriction. The cytosol fraction and renin granule fraction were prepared from the kidney cortex homogenate. Renin activity and the molecular weight of renin were measured by radioimmunoassay and by gel filtration, respectively. The intake of sodium-deficient diet for 4 weeks resulted in a 5-fold increase in plasma renin activity and led to a 2-fold increase in the renin activities of cytosol fraction and renin granule fraction. However, no significant change was observed in the protein content of the cytosol or renin granule fraction. The molecular weights of renin in the plasma, cytosol fraction and renin granule fraction from sodium-restricted rats were all 40000, being similar to those of control rats. In addition, no change in molecular weight or in activity was seen after acidification. These results indicate that active renin of regular size (M.W. 40000) is synthesized in the juxtaglomerular cells, stored in granules, and secreted into circulating blood following chronic sodium restriction.

**Keywords**—renin activity; molecular weight of renin; acid-activatable form of renin; inactive renin; rat plasma; kidney cortex; cytosol fraction; renin granule fraction; acidification; sodium restriction

It is generally accepted that renin is synthesized and stored in the juxtaglomerular cells. The results of numerous studies indicate that sodium intake is a major determinant in controlling the renin content of kidneys. It has been shown that alterations in the plasma renin activity reflect changes in the renal secretion of the enzyme. In recent years, multiple forms of renin, *e.g.*, renins with various molecular weights, and active and inactive renins, have been found in the plasma and kidney of humans and experimental animals.<sup>1)</sup> In addition, Poulsen *et al.*<sup>2)</sup> have demonstrated that high molecular weight (50000) renin is synthesized as the precursor of the enzyme in the mouse kidney.

In the previous paper,<sup>3)</sup> we reported the increased activity of renin in the plasma and storage granules of rat kidney cortex as a result of chronic sodium restriction. The present study was undertaken to determine whether or not the high molecular weight renin or acid-activatable form of renin (inactive renin) is newly synthesized in the kidney of rats under sodium restriction.

### Materials and Methods

**Animal Experiments**—Male Wistar rats weighing 120–130 g were used. For 1 week prior to the experiments, the rats were fed a standard laboratory rat chow (Oriental Yeast Co., MF) and provided with tap water *ad libitum*. The animals were separated into control and sodium-restricted groups. The sodium-restricted animals were maintained on a sodium-deficient diet and distilled water for 4 weeks, while the control animals were given a standard laboratory diet and tap water. The sodium-deficient diet (3.45 meq of sodium/kg) and standard laboratory diet (172 meq of sodium/kg) consisted of the same components as described in our previous paper.<sup>3)</sup>

**Processing of Blood Samples and Preparation of the Cytosol Fraction and Renin Granule Fraction**—The peritoneal cavity was opened under pentobarbital anesthesia (40 mg/kg, *i.p.*), the renal artery and vein were ligated, and both kidneys were removed and immediately cooled. An arterial blood sample was withdrawn through the aorta with a syringe, immediately cooled, and centrifuged at 3000 rpm for 15 min at 4 °C. The plasma was stored at -20 °C until analyzed. Each kidney was weighed, and the cortex was removed from the medulla, sectioned into thin slices, rinsed thoroughly with cold physiological saline to remove as much blood as possible, and homogenized with 0.45 M sucrose (1:8, w/v). The cytosol fraction and renin granule fraction were prepared from the homogenate according to the method described previously.<sup>3)</sup> The soluble renin from the granules was prepared according to the method of Funakawa *et al.*<sup>4)</sup>

**Determination of Molecular Weight of Renin**—The molecular weight of renin was estimated by gel filtration. A portion of the 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> 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 the use of blue dextran, and bovine serum albumin (M.W. 67000), ovalbumin (M.W. 45000), α-chymotrypsinogen A (M.W. 25000) and cytochrome c (M.W. 12900) were used as standards for molecular weight.

**Acidification**—Aliquots of renin-containing samples 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. As a control, a sample was dialyzed separately against 40 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at 4 °C for 40 h.

**Assays of Renin Activity and Protein Contents**—Renin-containing samples were incubated with partially purified rat renin substrate.<sup>5)</sup> Disodium ethylenediaminetetraacetic acid (20 mM), 8-hydroxyquinoline sulfate (1.6 mM), dimercaprol (4.0 mM) and diisopropylfluorophosphate (1.0 mM) were added to the incubation medium to inhibit converting enzyme and angiotensinases. After incubation for 15 min at 37 °C, the mixture was placed in a boiling water for 5 min to terminate the renin reaction. Angiotensin I (AI) generated in the incubation mixture was determined by radioimmunoassay<sup>6)</sup> using the CEA-IRE-SOLIN kit. The protein contents of fractions were determined by the method of Lowry *et al.*<sup>7)</sup>

**Statistical Analysis**—Student's *t*-test was used to determine whether differences between the control and sodium-restricted groups were significant. Differences were not considered significant if *p* > 0.05.

## Results and Discussion

Throughout the experimental period, no significant difference could be detected in average body weight or kidney weight per 100 g of body weight between the control and sodium-restricted rats. In the control rats, there were no significant changes in the renin activities of plasma, cytosol fraction and renin granule fraction during 4 weeks. The animals maintained on a sodium-deficient diet showed a marked increase in the renin activity of plasma (about 5 times), cytosol fraction (about 2 times) and renin granule fraction (about 2 times), in comparison with those of control rats (Table I). However, the protein contents in the latter two fractions remained unchanged. These results indicate that renin synthesis in the kidney is selectively stimulated by sodium restriction, as shown in the previous papers.<sup>3,8,9)</sup>

TABLE I. Effect of Acidification on Renin Activity in the Plasma, Cytosol Fraction, and Renin Granule Fraction from Control and Sodium-Restricted Rats

Dialysis	Plasma		Cytosol fraction		Renin granule fraction	
	Control	Sodium-restricted	Control	Sodium-restricted	Control	Sodium-restricted
	Renin activity <sup>d)</sup> (ng AI · ml <sup>-1</sup> · h <sup>-1</sup> )		Renin activity <sup>e)</sup> (μg AI · ml <sup>-1</sup> · h <sup>-1</sup> )			
Non-treated	11.3 ± 1.98	50.1 ± 3.47 <sup>c)</sup>	40.7 ± 6.42	76.7 ± 5.99 <sup>b)</sup>	2.87 ± 0.47	7.27 ± 0.61 <sup>b)</sup>
pH 7.4/7.4	11.9 ± 1.21	50.8 ± 2.76 <sup>c)</sup>	38.2 ± 8.51	75.1 ± 10.1 <sup>a)</sup>	3.21 ± 0.62	5.68 ± 0.28 <sup>b)</sup>
pH 3.0/7.4	12.0 ± 1.73	58.0 ± 4.73 <sup>c)</sup>	40.5 ± 7.61	81.2 ± 12.1 <sup>a)</sup>	3.29 ± 0.87	7.14 ± 0.26 <sup>b)</sup>

Values are means ± S.E. of four separate experiments. a), b), c) Values are significantly different from the control value (a) *p* < 0.05, b) *p* < 0.01, c) *p* < 0.001. d) Renin activity was expressed as ng of AI per ml of plasma per hour. e) Renin activity was expressed as μg of AI per ml of cytosol fraction and renin granule fraction per hour.

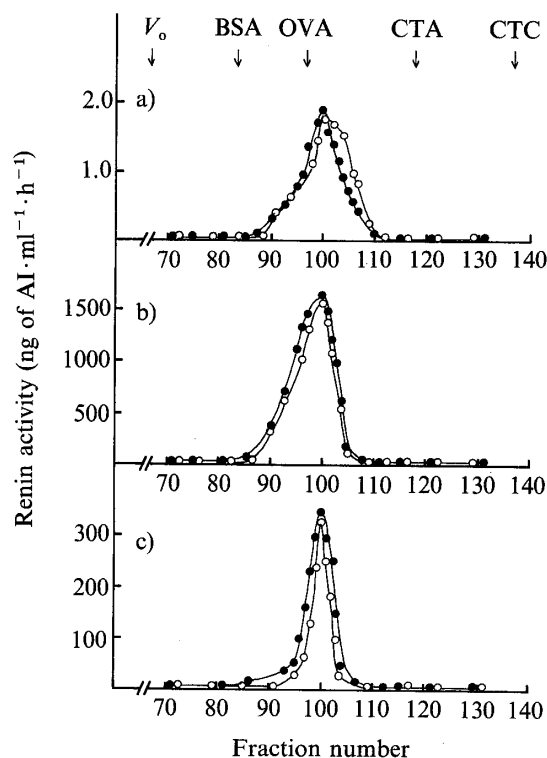


Fig. 1. Gel Filtration Profiles of Renin in the Plasma (a), Cytosol Fraction (b), and Renin Granule Fraction (c) on Sephadex G-100

A portion (0.5–2.0 ml) of each sample was applied to a Sephadex G-100 column. Renin activity was measured before (○) and after (●) acidification of each sample. The elution positions of molecular weight standards are indicated by arrows; void volume ( $V_0$ ), bovine serum albumin (BSA, M.W. 67000), ovalbumin (OVA, M.W. 45000),  $\alpha$ -chymotrypsinogen A (CTA, M.W. 25000) and cytochrome c (CTC, M.W. 12900). Renin activity was expressed as ng of AI per ml of fraction per hour.

In general, various enzymes, hormones and other physiologically active proteins are synthesized as inactive precursors, which are converted to the active form. Recently, renin precursor, which was a larger molecular weight than active renin of regular size (M.W. 40000), was identified in the cell-free system of the mouse kidney.<sup>2)</sup> In the present study, we checked the molecular weight of renin in the cytosol fraction and renin granule fraction from sodium-restricted rats by gel filtration. As shown in Fig. 1, renin activities in these fractions each showed a single peak with a molecular weight of 40000. Similarly, plasma samples contained only active renin with the same molecular weight.

On the other hand, several investigators<sup>10,11)</sup> have reported that an acid-activatable form of renin is present in the plasma and kidney extract of normal humans. Although the physiological role of this acid-activatable form of renin is not completely understood, the plasma values of active and inactive renins were shown to differ under various pathological conditions.<sup>12)</sup> In normal rats, Morris and Johnston<sup>13)</sup> observed the presence of an acid-activatable form of renin in granules, whereas Lauritzen *et al.*<sup>14)</sup> failed to find the acid-activatable form of renin in the kidney cortex. We observed only active renin of regular size in the cytosol and renin granule fractions from control rats, as demonstrated by Takaori *et al.*<sup>15)</sup> In the present study, we examined whether or not an acid-activatable form of renin was induced under conditions of sodium restriction. As shown in Table I, we could not detect an acid-activatable form of renin in the plasma, cytosol fraction or renin granule fraction. In addition, no significant changes in molecular weight (Fig. 1) or renin activity (Table I) were observed in the above three samples after acidification.

These findings indicate that even under conditions of increased synthesis of renin as a result of sodium restriction, only active renin of regular size is synthesized in the kidney and secreted into the circulating blood, as is the case in normal rats.

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