Factors Affecting Molecular Weight Conversion of Renin

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Our recent findings on molecular weight conversion in the case of renin are summarized and reviewed herein. The molecular weight of renin extracted from isolated renin granules from dogs and rats is approximately 40,000. This renin reacts with a constituent of kidney extract to form a high molecular weight renin of approximately 60,000. This constituent was termed a *renin binding substance*. This substance is probably contained in the cytosol of kidney cortex, and its characteristics are (1) heat instability, (2) non-dialyzability, (3) loss of binding ability by acidification at pH 3.0 and (4) apparent molecular weight is over 47,000. The binding reaction is mediated in two different ways; thiol oxidation with sodium tetrathionate and exposure of the reaction mixture at 0°C for several days without thiol oxidation.

BIG renin, which has a higher molecular weight than the renin of approximately 40,000 has been documented since the early 1970's.¹⁻⁴ Although the physiological function has not been totally elucidated, nevertheless, a study of "molecular weight of renin" should provide meaningful information on the renin-angiotensin cascade.

Early workers often assumed the high molecular weight renin might be a storage form, a proenzyme to be converted into a low molecular weight renin by limited proteolysis through mechanisms for renin release. Recently, however, Funakawa et al.⁵ reported that molecular weight of renin extracted from isolated renin granules in the dog was of a low molecular weight type. Our succeeding studies were focused on how the high molecular weight renin is formed and the relation to a living tissue.

Key Words:

Renin
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High molecular weight renin

In this paper we review our recent findings with regard to factors affecting molecular weight conversion of renin.

1. Molecular weight of renin in dog kidney extract and isolated renin granules

Kidney extract, a soluble part of homogenate of kidney cortex, is the most often used material for study on renin. However, renin is stored in renin granules in juxtaglomerular cells. From this aspect the kidney extract does not reflect the storage form of renin. Isolated renin granules are therefore required for a molecular studies involving renin.

In previous work,⁵⁻⁹ renin granules were isolated by discontinuous sucrose density gradient centrifugation¹⁰ or by passing through a membrane filter.⁷ In both methods, the kidney cortex was gently homogenized using a Potter-Elvehjem homogenizer with a loose-fitting pestle to minimize destruction of the cellular organelles. The homogenate was then centrifuged at 500 g for 10 min and the supernatant served for isolation of renin granules. The homogenate was separately centrifuged at 100,000 g for 60 min and

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Fig.1. Separation of *renin binding substance* from the kidney cortex with Sephadex G-75 column chromatography. 10% homogenate of kidney cortex was centrifuged to separate the cytosol. Two milliliters of cytosol was applied to the column and the eluates were separated into three fractions, A, B and C, each of which was concentrated to 2 ml and then mixed with renin in the granules and sodium tetrathionate. The protein concentration is shown by the solid line and the renin activity (M.W. 40,000) by the dotted line (left). The mixture of renin and A showed a typical elution profile of 60,000 (solid line), while renin and B or C showed a profile of 40,000 (dotted line) (right).

the supernatant served as the "cytosol" fraction.

Molecular weight of the renin in the kidney extract and in the granules was determined by gel filtration on a Sephadex G-100 column. Renin activity in the kidney extract appeared in the molecular weight region of 40,000 but when 5 mM sodium tetrathionate, a potent thiol oxidant, was added to the extract 30 min before application to a Sephadex column, the enzyme activity appeared in the molecular weight region of 60,000 and the activity at 40,000 disappeared. This 60,000 renin was changed to the 40,000 renin with addition of 50 mM dithiothreitol. Thus, the 60,000 renin was extractable from the kidney extract with thiol oxidation, and the 60,000 renin was reconverted into the 40,000 renin with reduction of disulfide bonds in the extract.

On the other hand, molecular weight of renin in the granules of the dog was approximately 40,000 and this weight did not change regardless of whether or not sodium tetrathionate was added. Accordingly, the molecular weight of renin does not alter itself and a *renin binding substance* may exist in the kidney extract, presumably cytoplasm, to react with the 40,000 renin to form the 60,000 renin.

2. Characteristics of renin binding substance

For a better comprehension of the character-

istics of the *renin binding substance*, the above mentioned cytosol fraction was utilized. One milliliter of the cytosol (8–9 mg protein) was mixed with renin in the granules (5,000 ng angiotensin I/hr) and 5 mM sodium tetrathionate, and after standing at 0°C for 30 min, the mixture was applied to a Sephadex column. The peak of renin activity appeared in the molecular weight region of 60,000, thus indicating that the *renin binding substance* was contained in the cytosol.

The cytosol was heated at 100°C for 10 min before mixing with renin in the granules. Acidification (dialysis against pH 3.0 Glycine-HCl buffer for 24 hr followed by dialysis against pH 7.2 phosphate buffer for 24 hr) of the cytosol was also performed before mixing with renin in the granules. Both heated and acidified cytosols failed to react with the 40,000 renin, while neutrally dialyzed cytosol (pH 7.2 for 48 hr) as control, reacted with the 40,000 renin to form the 60,000 renin.

Two milliliters of cytosol (16–18 mg protein) was fractionated by Sephadex G-75 column (1.6 cm × 90 cm) and was separated into three parts: fraction A: molecular weight of over 47,000, fraction B: from 47,000 to 32,000, fraction C: from 32,000 to 15,000. Each fraction was concentrated through a Diafilter membrane G-10T (Bioengineering Corp., Tokyo) and mixed with

Reaction mixture		Product
Rat renin (40,000)	Rat RBS	Big renin (60,000)
Dog renin (40,000)	Dog RBS	Big renin (60,000)

Fig.2. A scheme of compatibility of renin and *renin binding substance* (RBS) from the dog and the rat. Renin in the granules of the rat (M.W. 40,000) reacted with fraction A of both species to form the 60,000 renin. Dog renin (M.W. 40,000) also reacted with fraction A of both species. The reaction required 5 mM sodium tetrathionate as a thiol oxidant.

renin in the granules (10,000 ng angiotensin I/hr) and 5 mM sodium tetrathionate. Estimation of the molecular weight showed that fraction A, not B or C, led to the conversion of renin into the 60,000 form (Fig. 1). Thus, we concluded that characteristics of the *renin binding substance* were (1) heat instability, (2) non-dialyzability, (3) loss of binding ability by acidification and (4) apparent molecular weight is over 47,000.

3. Molecular weight conversion of renin in the rat

Renin from isolated renin granules of rats was examined for molecular weight by gel filtration, the mode of molecular weight conversion of renin being compared with that in the dog. The renin in granules was eluted estimated to be 40,000 either in the presence or absence of sodium tetrathionate. The renin in the kidney extract was also 40,000 while an addition of 5 mM sodium tetrathionate to the extract resulted in conversion of the renin to a molecular weight of 60,000.

Fraction A, separated from cytosol of the rat kidney cortex in the same manner as in the dog, reacted with renin in the granules of the rat to form the 60,000 renin, indicating that the *renin binding substance* of the rat also had a molecular weight of over 47,000. Moreover, fraction A of the rat reacted with renin in the granules of the dog.¹¹ Mixing fraction A of the rat (approximately 16 mg protein) with the dog renin (10,000 ng angiotensin I/hr) and sodium tetrathionate followed by gel filtration resulted in the appearance of a single peak of renin activity having a molecular weight of 60,000. When fractions A of the dog and the rat renin were mixed, the

result was the same (Fig. 2). Therefore, rat renin does bind with dog *renin binding substance* and *vice versa*. Thus, molecular weight of renin in the granules and the mode of the conversion in the rat closely resembles findings in the dog. The *renin binding substance* is required for the conversion mechanism in both species, and the reaction of renin with *renin binding substance* seems to be less species-specific, at least between the dog and rat.

4. Occurrence of molecular weight conversion of renin at low temperature

Molecular weight of conversion of renin in the kidney extract was due to thiol oxidation caused by addition of a particular oxidant, e.g., sodium tetrathionate. Nevertheless the conversion of renin in the extract was only recently found to occur during exposure at 0°C for about 7 days.⁹ Actually, the kidney extract was stored in a flask at 0°C for 7–8 days. When the molecular weight of renin in the stored extract was examined using gel filtration, the peak of renin activity appeared in the molecular weight region of 60,000. This exciting finding led to the further examination of the time-dependent changes in molecular weight of renin during cold storage. Our results indicated that a small amount of the 60,000 renin appeared on the 3rd day, an increase in amount of the 60,000 renin with a slight decrease in the 40,000 renin on the 5th day and the renin activity appeared exclusively at 60,000 on the 7th day.

Freezing the kidney extract at -80°C for 7 days produced no change in molecular weight of renin, suggesting that the reaction is probably repressed in a frozen state. Incubation at 37°C

for 3 hr also produced no change, indicating that the reaction is not facilitated by increasing the temperature. Such slow-reacting conversion was not due to oxidation of thiols, since the 60,000 renin was no longer re-converted into the 40,000 renin by addition of 50 mM dithiothreitol, and addition of 50 mM dithiothreitol to the extract did not inhibit the 40,000 renin from being converted into the 60,000 renin. This type of conversion was also found by Takaori et al.¹³ in rats and by Murakami¹² in hogs. The mechanism involved remain to be elucidated.

CONCLUSION

In conclusion, there is a strong possibility that high molecular weight renin is formed in the kidney. However, at least two conditions must exist for actual formation of the high molecular weight renin. First, the *renin binding substance* of extra-granular origin must directly come into contact with the renin. In this respect, the precise location of the *renin binding substance* in the kidney and whether morphological disorders in cortex allow renin to bind with the *renin binding substance* have to be elucidated. Second, binding reaction has to occur. In our examination, the reaction did not immediately take place either in the kidney extract or in the mixture of renin and the *renin binding substance*. One of factors facilitating the reaction proved to be thiol oxidation. Oxidation of thiols may occur, particularly in cases of toxicity or morphologically damaged tissue or in metabolic disorders, therefore, the oxidation seems to be a considerable factor involved in the conversion mechanism in living tissue.

Another factor evident with the cold storage yet not given physiological or pathophysiological significance, is the fact that the reaction automatically progresses with no addition of chemicals. In other words, the kidney extract intrinsically possesses the factors required for the conversion of renin. Whether or not the cold-induced conversion reflect the formation of high molecular weight renin in living tissue is the subject of ongoing studies in our laboratory.

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