Subcellular Distribution and Storage Form of Rat Renal Renin

GIUSEPPE SAGNELLA, PH.D., ROBERT PRICE, PH.D., AND WILLIAM PEART, F.R.S.

SUMMARY The subcellular distribution and nature of rat renal renin has been investigated by means of analytical subcellular fractionation and gel filtration on Sephadex G-100. During differential centrifugation, renin activity was recovered mainly in soluble and heavy mitochondrial fractions. On sucrose gradient centrifugation in either a conventional or in a B XIV zonal rotor, renin activity equilibrated at 1.54 M sucrose and was partially resolved from marker enzymes for mitochondria (succinate dehydrogenase), lysosomes (acid phosphatase), plasma membranes (alkaline phosphatase), and peroxisomes (catalase). On gel filtration of the soluble or extracts of the renin-granular fractions on Sephadex G-100, renin activity eluted as a single peak with an apparent molecular weight (MW) of 42,000; no change in activity was found when these fractions were acidified to pH 3.0. When kidney homogenates were prepared in the presence of the proteolytic inhibitor N-ethylmaleimide (NEM, 10 mM), whereas the renin from the granular fractions displayed a MW of 44,000, that from the soluble fraction was apparently higher (69,000). Addition of NEM (10 mM) to the soluble fraction previously shown to contain only the low MW form of renin also resulted in an apparently high MW form of renin.

These results indicate that rat renal renin is associated with a mechanically fragile, distinct type of subcellular organelle. Renin within this structure is of the low MW form and is not acid activatable. The soluble fraction, however, contains a factor(s) that, in the presence of NEM, combines with the low MW renin to form a complex of apparently high MW. (Hypertension 2: 595-603, 1980)

KEY WORDS • renin granules • renin zymogen • acid pH activation • N-ethylmaleimide • high molecular weight renin

WHILE it is now generally accepted that renal renin (E.C. 3.4.99.19) is synthesized and secreted by the granulated epithelial cells of the afferent glomerular arteriole,1 previous tissue fractionation studies2,3 have been contradictory as to the precise subcellular distribution of the enzyme. Also, limited information is available on the nature of the storage form of rat renin. It has been reported, however, that rat renin is stored as an inactive form that can be activated by acidification to pH 3.3,4 yet others5,6 have been unable to demonstrate acid activatable forms of rat renin.

Recently, Inagami et al.7 found that, in the presence of the proteolytic inhibitor N-ethylmaleimide, only a high molecular weight (HMW) form of renin (60,000) was extracted from the rat kidney; consequently they concluded that this form of renin represented a precursor of the low molecular weight (LMW) form of the enzyme. However, since these studies were carried out with crude soluble cortical extracts, the relationship between this HMW form of renin and the granular renin remained undetermined.

Therefore, it was thought of importance to investigate further the subcellular distribution of renin and in particular to determine the effect of acidification and N-ethylmaleimide on the activity and molecular size of renin not only from soluble but also from granular fractions.

Materials and Methods

Preparation of Cortical Homogenates

Male Wistar rats (180–200 g) that had free access to water and standard laboratory food were killed by cervical dislocation. The kidneys were removed, stripped of the capsule and bisected. The cortices were
Density Gradient Centrifugation of the Heavy Mitochondrial Fraction

A portion (2 ml) of the heavy mitochondrial fraction was layered on top of a freshly prepared discontinuous density gradient consisting of 1.6 M (7 ml), 1.5 M (7 ml) and 1.4 M (7 ml) and centrifuged at 80,000 g for 60 minutes in a 3 X 23 ml swing-out rotor on a zonal rotor with a Superspeed 65 Ultracentrifuge (MSE Ltd). Sucrose solutions (BDH Laboratory Reagents) were prepared in deionized water at room temperature and precooled to 4°C. Preliminary experiments were carried out to define optimal conditions for the separation of renin from the other marker enzymes; the procedure described below was eventually developed. This type of centrifugation exploited the versatility of the zonal rotor system, which allowed the contents to be moved in a radial direction intermittently to increase rupture of the particles, and centrifuged at 43,000 X g. After centrifugation, the contents of the rotor were fully separated into approximately 45 fractions (10 ml) by displacement with 60% (W/W) sucrose. All fractions collected were stored at -20°C, and enzymes, protein, and sucrose determinations were performed within 48 hours.

Gel Filtration on Sephadex G-100

Gel filtration was carried out at 4°C on Sephadex G-100 (particle size 40-120 μ) in a column with an internal diameter of 1.6 cm and length of 70 cm (Pharmacia Fine Chemicals, Uppsala, Sweden) eluted with 0.1 M sodium phosphate/sodium dihydrogen phosphate buffer pH 7.2 or 0.1 M sodium chloride/10 mM sodium pyrophosphate buffer pH 6.6, and fractions (2 ml) were collected at a flow rate of about 0.3 ml/min. The column was calibrated with blue dextran (MW 2 x 10⁶, Pharmacia Fine Chemicals), bovine plasma albumin (MW 68,000), egg albumin (MW 43,000), myoglobin (MW 17,500), and cytochrome c (MW 11,700) (all from Koch-Light Laboratories, Colnbrook, U.K.). All standards were eluted before and after the unknown samples, and apparent molecular weights were determined by the method of Andrews.11

Preparations of Samples for Gel Filtration

Renin was extracted from the granular fraction by osmotic shock treatment. The heavy mitochondrial fraction, prepared as described, was resuspended in 20 mM NaCl and left at 0°C for 60 minutes, vortexed intermittently to increase rupture of the particles, and centrifuged at 44,000 g for 20 minutes. A portion of the clear supernatant (4 ml) was used for gel filtration on Sephadex G-100.

When studying the effects of N-ethylmaleimide (NEM) on the gel filtration behavior of renin from the granular fraction, the heavy mitochondrial fraction was isolated and subjected to osmotic treatment exactly as described, with the exception that NEM (10 mM) was present throughout the whole procedure. Portions (4 ml) of the extract were used for gel filtration and eluted in the pyrophosphate buffer. In this series of experiments, a soluble fraction was also obtained from cortical homogenates prepared in 0.3 M sucrose or 10 mM sodium pyrophosphate/0.1 M sodium chloride with or without NEM (10 mM) by centrifugation at 80,000 g for 60 minutes. Portions (4 ml) of each supernatant were used for gel filtration on...
Sephadex G-100 immediately or after dialysis (for 24 hrs) in Visking cellophane tubing (8/32) against 200 volumes of the sodium pyrophosphate buffer at 4°C and eluted in the same buffer. In some experiments, homogenates were prepared in 20 mM NaCl/10 mM NEM and centrifuged at 80,000 g for 60 minutes to obtain the soluble fraction; portions (4 ml) were used for gel filtration and eluted in the sodium pyrophosphate buffer.

Acid pH Treatment of Eluates after Gel Filtration

Portions (2 ml) of the soluble fraction and of extracts of the heavy mitochondrial fraction prepared by osmotic treatment in 20 mM NaCl as described were applied to the Sephadex gel and eluted in 0.1 M sodium phosphate buffer, pH 7.2. Then portions (300 µl) of the fractions collected after gel filtration were adjusted to pH 2.8-3.0 by the addition of 50 µl of 0.5 M HCl; 50 µl of water was added to parallel neutral pH control samples. The tubes were left at 0°C for 60 minutes, and then 650 µl of 0.1 M sodium phosphate buffer pH 7.2 was added to all samples to give a final pH of 6.8-7.2 before assay for renin activity.

Acid pH Treatment of Soluble and Renin-Granular Fractions by Dialysis

Immediately after isolation, portions (2 ml) of the soluble fraction or of a renin-granular preparation prepared as described previously were dialyzed in Visking cellophane tubing (8/32) against 500 volumes of pH 3.0 buffer (0.05 M glycine-HCl/0.1 M NaCl) or pH 7.0 buffer (0.1 M sodium phosphate/0.1 M NaCl) for 20 hours. The samples were then transferred to pH 7.0 buffer (0.1 M sodium phosphate) and left for a further 20 hours before determination of renin activity.

Enzyme Assays

Renin activity was measured by radioimmunoassay determination of the angiotensin I generated from homologous plasma substrate. The recovery of angiotensin I during the incubation was determined by adding standard angiotensin I (25 ng) to the reaction mixture incubated without substrate and expressed as a percentage of control incubations without sample or substrate. Succinate dehydrogenase (Succinate: Acceptor oxidoreductase, E.C.1.3.99.1); acid phosphatase (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.2); alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.1) and catalase H2O2 (oxidoreductase, E.C.1.1.1.6.) were used as markers for mitochondria, lysosomes, plasma membranes, and peroxisomes respectively.

Succinate dehydrogenase was assayed by a minor modification of the procedure described by Pennington with p-nitrophenylphosphate as the substrate. Alkaline phosphatase was determined by the procedure of Shibko and Tappel and catalase as described by Baudhuin et al. Enzyme activities are expressed in units where 1 unit represents the amount of product formed in nmol/min/mg of protein or ml fraction for succinate dehydrogenase, acid, and alkaline phosphatase; and µmol hydrogen peroxide hydrolyzed per min/mg of protein or ml fraction for catalase. Renin activity units are defined in pmol angiotensin I produced per min/mg of protein or ml fraction.

Other Methods

Protein was determined by the method of Lowry et al. with bovine plasma albumin as a standard. The concentration of sucrose in the density gradient fractions was determined from the refractive index using an Abbe refractometer (Bellingham and Stanley Ltd., U.K.).

Results

Differential Centrifugation of Renal Cortical Homogenates

Renin activity was found in all fractions isolated, but the enzyme was maximally enriched in the heavy mitochondrial fraction (table 1), and the microsomal fraction contained less than 1% of the total activity of the homogenate.

The recovery of angiotensin I during the renin assay ranged from 93% to 100% in all fractions with the exception of the total homogenate, where it was 63%, indicating generally satisfactory inhibition of angiotensinase activity. Succinate dehydrogenase was enriched mainly in the heavy and light mitochondrial fractions, and little activity was recovered in the soluble and microsomal fractions. The specific activity of catalase was highest in the light mitochondrial fraction and in the soluble phase. Alkaline phosphatase was maximally enriched in the microsomal fraction, and little activity was detected in the soluble phase (less than 3% of the total). The rather high percentage of protein and enzyme activities in the nuclear fraction can be explained on the basis of incomplete disruption of the tissue as this fraction was heterogeneous in composition and, as demonstrated by microscopic examination, contained not only nuclei but also intact cells.

The nature of the sedimentable structure associated with renin was further investigated by density gradient centrifugation of the heavy mitochondrial fraction.

Density Gradient Centrifugation of the Heavy Mitochondrial Fraction

After centrifugation, the gradient consisted of a clear upper layer (F1), three visible particulate bands (F2, F3, and F4), the uppermost of which (F3) was the most dense, a clear zone (F5), and a visible yellow-brown pellet (F6), as shown in figure 1. Renin activity was recovered mainly in the particulate region (F2 and F3), which accounted for 80% of the total. The
Table 1. Enzyme and Protein Distribution in Renal Subcellular Fractions Prepared by Differential Centrifugation

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Fractions isolated</th>
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<tbody>
<tr>
<td></td>
<td>Homogenate</td>
</tr>
<tr>
<td>Renin</td>
<td>(a) —</td>
</tr>
<tr>
<td></td>
<td>(b) 3.4 ± 0.8</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>(a) —</td>
</tr>
<tr>
<td></td>
<td>(b) 103.6 ± 13.5</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>(a) —</td>
</tr>
<tr>
<td></td>
<td>(b) 12.4 ± 3.8</td>
</tr>
<tr>
<td>Catalase</td>
<td>(a) —</td>
</tr>
<tr>
<td></td>
<td>(b) 71.6 ± 7.7</td>
</tr>
<tr>
<td>Protein</td>
<td>(a) —</td>
</tr>
</tbody>
</table>

(a) Distribution of renin, selected marker enzymes and protein in the fractions isolated as a percentage of the total in all fractions. (Nuclear + heavy + light mitochondrial + microsomal + soluble).

(b) Specific activity of the enzymes in the homogenate and in the fractions isolated. Specific activity is expressed as units/mg of protein.

The fractions were isolated as described in Materials and Methods section and the results are the means ± se for three to four experiments.

The recoveries (%) were: renin 87.9 ± 13.2; alkaline phosphatase 95.6 ± 6.5; succinate dehydrogenase 88.5 ± 6.9; catalase 120.6 ± 15.0; and protein 85.8 ± 12.0.

Recovery of angiotensin I during the renin assay conditions ranged from 84% to 120% in all fractions, indicating that angiotensinase activity did not alter the observed distribution of renin.

Protein was found mainly in the upper particulate band (F2). This fraction also contained about 80% of the succinate dehydrogenase and alkaline phosphatase activities, whereas acid phosphatase and catalase were distributed broadly throughout the gradient (fig. 1).

While these results indicate that the renin activity of the heavy mitochondrial fraction was not a result of adsorption to other subcellular structures, only limited separation of renin from the other marker enzymes was obtained. A higher degree of resolution was, however, obtained using density gradient centrifugation in a B XIV zonal rotor.

Density Gradient Centrifugation of a Total Postnuclear Supernatant in a B XIV Zonal Rotor

After centrifugation, the gradient consisted of a clear yellow-brown layer at the least dense region of the gradient, which was followed by a white cloudy layer turning dense brown and becoming faintly cloudy up to fraction 20. After the second stage centrifugation, two major particulate bands were found; one from 42% to 45% (W/W) sucrose was faintly brown, and the other from 45.4% to 57.4% sucrose was yellow-brown in appearance. The overall recovery of protein and enzymes was generally satisfactory, ranging from 92% to 110% with the exception of succinate dehydrogenase which was usually lower (56%). The distribution of renin, protein, sucrose profile, and marker enzymes is shown in figure 2.

Renin activity was recovered predominantly in the clear yellow-brown layer (soluble phase), extending from 9% to 24% (W/W) sucrose, and in the particulate band equilibrating from 42% to 45% sucrose containing 36% and 30% respectively of the total recovered. At its maximum activity, at 45% sucrose, renin was enriched ninefold over the original homogenate. A smaller peak of renin activity was also found at 48% sucrose, but this contained only about 10% of the total. Protein was recovered mainly throughout the first 20 fractions (about 88% of the total) and in two minor peaks after the second stage centrifugation corresponding to the two particulate bands.

Alkaline phosphatase was located in a zone extending from 13.8% to 42.4% sucrose, representing 92% of the total activity. Succinate dehydrogenase was recovered mainly between 24% to 43% sucrose (88% of the total). Acid phosphatase was found predominantly in the soluble phase and in the particulate part of the gradient (45.4% to 57.4% sucrose) representing 65% and 20% of the total respectively. Catalase activity was similar in distribution to acid phosphatase.

Molecular Weight of Renin from Soluble and Granular Fractions

The reproducibility of elution volumes of standard proteins was satisfactory; in replicate experiments, these did not differ by more than 6%–10%. A single peak of renin activity was observed from the soluble fraction isolated from cortical homogenates prepared in sucrose (0.3 M), which eluted with a mean apparent molecular weight of 42,000 (range 39,000–45,000, n = 4). After the osmotic treatment of the particulate
fractions, about 85% of particulate renin was solubilized. After gel filtration on Sephadex G-100, a single peak of activity was found displaying a mean apparent MW of 42,000 (range 41,000–44,000, n = 4). Similar results were also obtained for renin extracted from renin granules prepared by zonal rotor centrifugation (n = 2).

Effect of Acidification on Renin Activity after Gel Filtration on Sephadex G-100

To determine the presence of HMW inactive forms of rat renin of the type described in pig kidney, the soluble phase and extracts of the heavy mitochondrial fraction were chromatographed on Sephadex G-100. Eluates from the void volume onwards were subjected to acidification. Renin activity in both the soluble or granular fractions eluted with an apparent MW of 42,000 and was not altered by the acidification treatment, as compared with neutral treated parallel samples (fig. 3).

No change in renin activity was found when the total soluble fraction and the renin-granular fractions were treated by dialysis at acid pH.

Effect of N-ethylmaleimide on Gel Filtration of Renin from Soluble and Granular Fractions

While renin from the soluble fraction isolated in the absence of NEM and dialyzed overnight (pH 7) before gel filtration eluted with an apparent MW of 43,000 ± 2300 (n = 3), when this fraction was prepared in the presence of NEM (10 mM) and immediately subjected to gel filtration, renin eluted entirely as a HMW protein (MW 69,000 ± 9000, n = 3), as shown in figure 4a.

When the heavy mitochondrial fraction was subjected to osmotic shock treatment in the presence of NEM (10 mM), about 90% of the granular renin was released into the extracellular & granulation, and on gel filtration renin eluted with an apparent MW of 44,000 ± 1100 (n = 3). When the soluble fraction was isolated in the medium used for osmotic disruption of the heavy mitochondrial fraction in the presence of NEM, the renin activity eluted as an apparently HMW protein (MW 64,000, n = 3, fig. 4b), showing that it was only formed in the presence of material in the soluble fraction.

These observations led to a further investigation of the effect of NEM on the gel filtration behavior of renin from the soluble fraction. When the soluble fraction was isolated in the pyrophosphate/sodium chloride buffer in the absence of NEM and stored at 4°C for 24 hours and then applied to the Sephadex gel, renin eluted as a LMW form (MW 46,000 ± 2300, n = 3, fig. 4c). However, when the soluble fraction was similarly isolated and stored at 4°C for 24 hours, and NEM (10 mM) was added 2 hours before gel filtration, the renin activity eluted entirely as the HMW form (MW 78,000 ± 14,000, n = 3, fig. 4c).

Figure 1. Density gradient centrifugation of the heavy mitochondrial fraction. Appearance of gradient and distribution of renin and marker enzymes. After centrifugation, six fractions were collected. Thick lines in tube indicate the position of visible bands. The distribution of enzymes and protein (ordinate) is given as a percentage of the total recovered in the gradient in each of the fractions isolated. The results are the mean values of six experiments for renin and protein, and of four for the other enzymes. Recoveries of protein and enzymes ranged from 78% to 92%. (a) Renin = -----; protein = ······. (b) Succinate dehydrogenase = ······; acid phosphatase = ······. (c) Catalase = ······; alkaline phosphatase = ······.
FIGURE 2. Density gradient centrifugation of a total postnuclear supernatant in a B XIV zonal rotor. Distribution of renin, selected marker enzymes, protein and sucrose concentration (ordinate) in each of the fractions isolated (1 to 60). Enzyme profiles are expressed in units/ml fraction, protein in mg/ml fraction, and sucrose as % W/W. The results are from one representative experiment (n = 4). Details of preparative procedure are given in Materials and Methods section. Top Panel: Renin = ————; protein = ————. Middle Panel: Catalase = ————; alkaline phosphatase = ————. Bottom Panel: Succinate dehydrogenase = ————; acid phosphatase = ————. Sucrose is given by the continuous line.
STORAGE FORM OF RAT RENIN/Sagnella et al. 601

Figure 3. Distribution of renin activity after gel filtration of the soluble and heavy mitochondrial fraction on Sephadex G-100. Effect of acidification. (a) Heavy mitochondrial. (b) Soluble fraction. These were isolated and subjected to gel filtration as described in the text. Eluates after gel filtration were acid (open triangle) or neutral pH (black circle) treated before determination of renin activity as described in Materials and Methods.

Figure 4. Distribution of renin activity after gel filtration of soluble and granular fraction on Sephadex G-100. Effect of N-ethylmaleimide. (a) Soluble fraction from cortical homogenates prepared in 10 mM sodium pyrophosphate/0.1 M sodium chloride buffer (pH 6.6) and dialyzed (4°C, 20 hours) before gel filtration (open squares, dashed line); soluble fraction isolated in the same buffer containing N-ethylmaleimide (NEM, 10 mM) and applied directly to the gel (open squares/solid line). (b) Heavy mitochondrial fraction prepared in the presence of NEM (10 mM) and extracted by osmotic lysis as described in the text before gel filtration (black circles, solid line); soluble fraction from cortical homogenates prepared in 20 mM NaCl/10 mM NEM (black circles, dashed lines). (c) Soluble fraction prepared in sodium pyrophosphate/sodium chloride buffer and left at 4°C for 20 hours before gel filtration (open triangles, dashed line); soluble fraction isolated in sodium pyrophosphate/sodium chloride buffer, left at 4°C for 20 hours and then NEM (10 mM) added two hours before gel filtration (open triangles, solid line). Details of preparation of soluble and heavy mitochondrial fraction are given in Materials and Methods section. V₀ = void volume of column. The recoveries of renin from the column ranged from 80% to 90%.
Subcellular Distribution of Rat Renin

During differential centrifugation, renin activity was recovered predominantly in the heavy mitochondrial fraction, suggesting that the enzyme was associated with a sedimentable particle similar in size to renal mitochondria. In contrast, the microsomal fraction, which in rat kidney was also enriched in brush-border membranes fragmented to microsomal size as indicated by the increased specific activity of alkaline phosphatase (table 1), contained little renin activity. Although these brush-border membranes are rich in peptidases, which can hydrolyse angiotensin I, the fact that the recovery of angiotensin I during the renin assay was satisfactory excludes this possibility as an explanation of the low renin activity. Additionally, since the microsomal fraction was not washed with hypotonic salt solutions, the presence of renin activity in this fraction may have been a result of entrapped soluble material, in view of the higher renin content in the soluble fraction (table 1). Although the renin activity in the soluble fraction could originate from organelles disrupted during the homogenization of the renal tissue, the results from the differential centrifugation experiments cannot exclude the possibility that the renin activity was located entirely in the soluble phase and that its presence in the heavy mitochondrial fraction was due to absorption on a sedimentable structure such as mitochondria.

While other workers have investigated the subcellular distribution of renal renin using entirely the technique of differential centrifugation, and concluded that renin was associated with mitochondria or lysosomes, the inherent poor resolution of this technique makes it difficult to allocate a given enzyme to a particular organelle. In the present study, therefore, the subcellular distribution of renin was investigated further by subjecting the heavy mitochondrial fraction to density gradient centrifugation in a conventional rotor and a total postnuclear supernatant to gradient centrifugation in B XIV zonal rotor. This latter procedure overcame the poor resolution of differential centrifugation and also the limited load capacity of gradient centrifugation in conventional swing out rotors. During density gradient centrifugation, renin displayed a distribution distinct from selected marker enzymes for mitochondria, lysosomes, brush-border membranes, and peroxisomes. The renin was thereby enriched about ninefold over the original homogenate at its peak activity in the part of the density gradient corresponding to 1.5-1.6 M sucrose. Similar results have also been described not only for rat but for dog and rabbit renal renin. Whereas on gradient centrifugation of the heavy mitochondrial fraction a broad peak of renin activity was observed, during density gradient centrifugation of the total postnuclear supernatant in the B XIV zonal rotor, the particulate renin was clearly separated from the other marker enzymes (fig. 2). Under these conditions, while the bulk of the particulate renin equilibrated in the region of the gradient corresponding to 45% (W/W) sucrose, a smaller peak of renin was also found at 48% sucrose. This is of particular interest since it suggests the existence of more than one type of renin particle. On the other hand, this may merely represent adsorption of renin solubilized during the initial homogenization of the tissue onto the subcellular organelles also present in this region of the density gradient. Gross and Barajas in their study on the large scale isolation of renin granules from the rabbit renal cortex using a SZ-14 re-orienting zonal rotor described a similar, though less efficient, separation of renin from the other subcellular organelles as based on enzymatic criteria (fig. 2).

These results, therefore, indicate that renal renin is associated with a labile subcellular organelle which during differential and density gradient centrifugation behaves differently from mitochondria, lysosomes, brush-border membranes, and peroxisomes of the tubular cells, and hence support the opinion that the enzyme is located within the specialized granules of the juxtaglomerular apparatus.

Storage Form and Interaction of Rat Renin with N-Ethylmaleimide

When renin was extracted from the granular fraction by osmotic lysis, it was exclusively of the LMW form (MW 42,000), and its activity was not increased by acidification to pH 3.0 (fig. 3). Renin from granular structures of the dog also displayed a MW of about 40,000, indicating that in these species the storage form of renin is of the LMW type. In the present studies, the possibility of proteolytic conversion of renin by enzymes from other subcellular organelles was minimized by the rapid isolation of subcellular organelles and the subsequent osmotic lysis at 0°C before gel filtration on Sephadex G-100. This molecular weight value is in agreement with that obtained for purified renin isolated from the rat and pig cortical homogenates.

As described in this paper, absence of acid activatable forms of renin in rat kidney extracts has been reported by other workers; in contrast, Morris and Johnston found that the renin activity from a granular fraction of the rat kidney could be activated by acidification to pH 3.3. Although in our study the molecular size of renin from the granular fraction was similar to that described by these workers (MW 44,000), we were unable to detect an increase in activity on acidification (fig. 3). Since the acidification procedure described here has been used to detect acid-activatable HMW renin in pig kidney (Boyd and Johnston), the absence of an increase in renin activity under these conditions clearly indicates that rat kidney is devoid of this form of renin activity. Therefore, the difference between our results and those of Morris and Johnston might be explained by methodological differences such as the use of heterologous renin substrate by these workers. Another possibility concerns the physiological state of the rats, and it may be relevant that acid activation of renin in rat cortical extracts was...
demonstrated after stimulation of renin formation induced by bilateral adrenalectomy and salt depletion. The possibility of proteolytic degradation of renal renin during the isolation procedure has also been considered, in particular, to determine the subcellular distribution of the HMW form of renin found by previous workers when cortical soluble extracts were prepared in the presence of NEM. In the present study we have found that the presence of NEM induced the formation of an apparently HMW form of renin (fig. 4), which was restricted to the soluble fraction and was not found when the granular fraction was prepared in the presence of this agent. While Inagami et al. concluded that this HMW form of renin represented a precursor of the LMW form, further experiments reported here (fig. 4c) do not support this conclusion. Addition of NEM to the soluble fraction previously demonstrated to contain only the LMW renin still resulted in renin eluting as HMW form (fig. 4c), so our results suggest that NEM induced the formation of a renin-protein(s) complex eluting with an apparent increase in molecular weight. This is supported by the observation that NEM did not alter the elution behavior of renin from the granular fraction, which also excludes the possibility of alteration in configuration of the renin molecule or formation of a renin-dimer.

While the material is restricted to the soluble phase, its nature is unknown. Furthermore, it remains to be clarified whether thiol or amino groups are involved since NEM can react with both type. Funakawa et al. reported similar findings in dog kidney and more recently, Kawamura et al. isolated from dog kidney soluble extract a protein preparation representing a precursor of the LMW type and its activity is not increased by acidification. The soluble fraction, however, contains not only LMW renin but also factor(s) that in the presence of NEM induce the formation of an apparently HMW form of renal renin.

In conclusion, the storage form of rat renin is of the LMW type and its activity is not increased by acidification. The soluble fraction, however, contains not only LMW renin but also factor(s) that in the presence of NEM induce the formation of an apparently HMW form of renal renin.

References
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Hypertension. 1980;2:595-603
doi: 10.1161/01.HYP.2.5.595

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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