Inactive Renin of High Molecular Weight (Big Renin) in Normal Human Plasma

Activation by Pepsin, Trypsin, or Dialysis to pH 3.3 and 7.5

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SUMMARY Normal plasma contains inactive renin, which becomes active when plasma is dialyzed to pH 3.3 and to pH 7.5, or treated with pepsin or trypsin. Under optimal conditions, each of these procedures activated the same quantity of renin, which was not further increased by repeating or combining two procedures, thus suggesting that the same pool of inactive renin was activated by each procedure. When plasma was fractionated by gel filtration, dialysis activated very little renin in eluates. Trypsin activated renin, but under some conditions also destroyed renin. Pepsin fully activated the inactive renin in eluates without evidence of destruction of renin. The pepsin-activated renin of normal plasma eluted from Sephadex G-100 in a peak of apparent molecular weight (MW) 58,000 and from Sephacryl S-200 with apparent MW 53,000, like big renin in plasma of patients with diabetic nephropathy. Inactive renin was usually increased in amount in plasma of sodium-depleted normal men, but the elution volume did not change with sodium intake. When renin was fully activated in plasma incubated with pepsin or trypsin, the apparent MW of the main peak of big renin did not change appreciably. Inactive renin in plasma was usually increased after sodium depletion, but the elution volume did not change. Active renin of normal plasma had an apparent MW near 41,000 on both gels. Thus, we conclude that big renin is present in normal plasma in amounts at least equal to and usually greater than active renin (the ratio depending on sodium intake) and that pepsin activation readily demonstrates big renin in eluates from gel filtration. (Hypertension 2: 750-756, 1980)

KEY WORDS • big renin • plasma renin • activation • pepsin • trypsin

When plasma is dialyzed to pH 3.3 and then back to pH 7.5, or incubated with trypsin or pepsin, or allowed to stand for several days at −4°C, renin activity increases due to the appearance of an enzyme closely resembling active renin.¹ The source of this active enzyme generated in vitro is referred to as "inactive renin" or "prorenin." Although inactive renin is readily demonstrated in plasma subjected to dialysis (pH 3.3 → 7.5), dialysis of eluates from gel filtration of normal plasma activates only a small fraction of inactive renin.¹ Dilution of plasma impairs activation by dialysis (pH 3.3 → 7.5) and contributes to the poor recovery of inactive renin in dialyzed eluates from gel filtration.⁷ Nevertheless, an inactive renin of higher molecular weight than active renin has been demonstrated in plasma of normal volunteers on a high sodium intake; salt-loading suppresses active renin and facilitates the demonstration of the inactive renin.⁵⁶

In the present report, we describe the activation of renin in fractions of normal plasma by proteases. Incubation with pepsin at pH 3.3 fully activated the inactive renin in diluted plasma or eluates from gel filtration. The effect of trypsin on renin was more complex, involving both activation and destruction. Using these methods, we show that the inactive renin in normal plasma has a higher molecular weight than that of active renin.
Materials and Methods

Subjects

Eighteen volunteers (13 men and 5 women), aged 19 to 40 years, were studied after giving informed consent. Medical history and physical examination were normal in 16 of the subjects; in two cases, a slight elevation of blood pressure was noted on one or more occasions. All subjects had normal urinalysis and plasma urea, creatinine, and electrolytes (sodium, potassium, bicarbonate, and chloride). Each volunteer was studied after 4 days on a low-sodium diet (100-542 mEq/day) and also after taking an unrestricted diet (80-170 mEq sodium/day). Ten subjects received added sodium chloride (total sodium intake, 200-250 mEq/day). At the end of each period, blood was drawn and a complete day's urine was collected to verify compliance with the diet. In all cases, blood was drawn at noon after a morning of ordinary activity. In four cases, samples were also drawn at 8:00 a.m. before arising.

Methods

Plasma Renin

Plasma renin activity (PRA) was measured by Haber's method.19 Active renin was measured by radioimmunoadsorbent assay of angiotensin I generated during incubation with added sheep angiotensinogen.6,7 Active plus inactive renin was measured similarly in plasma dialyzed to pH 3.3 and 7.5 at 4°C.

Plasma was incubated with trypsin at pH 7.5 and −4°C for 10 minutes. Trypsin (crystallized type III, Sigma) was dissolved in buffer,8 and 10 μl was added to 100 μl of sample to make the trypsin concentration 1 mg (10,000 BAEE units)/ml plasma, or 0.1 mg/ml for eluates from gel filtration. The reaction was stopped by adding lima-bean trypsin inhibitor (type II-L, Sigma) in the same volume and concentration as trypsin. (This quantity of LBTI was more than twice the amount necessary to inactivate the trypsin present, as judged by complete inhibition of esterolytic activity after addition of LBTI.) Sheep angiotensinogen was added before incubation at 37°C and radioimmunoassay of angiotensin I.

Pepsin (crystallized, Sigma) was dissolved in buffer pH 3.3,8 and 5 μl was added to 100 μl plasma or eluate previously dialyzed to pH 3.3 to give a pepsin concentration of 50 μg (150 units)/ml. After incubation at 32°C for 30 min, the incubates were chilled and dialyzed to pH 7.5 at 4°C before addition of sheep substrate and assay of renin.7 These conditions were found to yield the best recovery of inactive renin in plasma containing large amounts of big renin.11

Gel Filtration

Columns of Sephadex G-100 and Sephacryl S-200, 2.5 × 85 cm, were packed with 0.15 M sodium-chloride-phosphate buffer, pH 7.5. Then 2 ml of plasma, to which ovalbumin 40 mg was added, were placed on the column. Buffer was run through the columns at a rate of 0.2 ml/min; 3 ml fractions were collected. Each column was calibrated with pure proteins (human serum albumin, ovalbumin, and either α-chymotrypsinogen or transferrin, Sigma). As human serum albumin was present in all runs of plasma, its elution volume was noted in every run, together with that of ovalbumin, which lags behind the plasma proteins and can thus be detected in low concentrations added to each plasma sample. The regular observation of elution volume of these two internal standards was used to detect small variations of running rates when the column was used repeatedly. The elution characteristic of each protein was defined by its partition coefficient, \(K_{av}\). Columns eluates were assayed for active renin.

Recovery of active renin from the column was estimated as the percent of the active renin in unmodified plasma. Inactive renin in the column eluates was activated by dialysis (pH 3.3 → 7.5), trypsin (0.1 mg/ml), or pepsin (50 μg/ml) as previously described. Inactive renin was calculated by subtracting active renin from total renin in activated plasma. Recovery of renin activated by each procedure was calculated by comparing the renin activated in all similarly treated eluates with the inactive renin in plasma. An aliquot of each treated eluate was assayed before incubation with sheep substrate to detect preformed angiotensin I or similar immunoreactive material; and another aliquot was checked for binding of tracer in the absence of antibody.

Significant “blanks” (i.e., immunoreactive A-I-like material found prior to incubation with angiotensinogen) were observed only in pepsin-treated eluates containing human angiotensinogen. Pepsin is known11 to produce angiotensin I from angiotensinogen at pH less than 6.5. Human angiotensinogen eluted from Sephadex G-100 and Sephacryl S-200 just before serum albumin. When human plasma fractions eluting before the albumin peak were incubated with pepsin, immunoreactive angiotensin I was formed. Like angiotensin I, the immunoreactive material was largely, but not completely removed by dialysis for 24 hrs at 4°C against buffer pH 7.5. Blanks were far higher in eluates neutralized, but not dialyzed. The small amount of residual blank after dialysis was measured before each protease-treated eluate was incubated with sheep angiotensinogen, and the blank was subtracted from total angiotensin before calculation of renin activity.

In eluates containing peak concentrations of inactive renin, the preformed A-I blank was between 0 and 5% of the A-I generated during incubation with angiotensinogen. In the eluates containing the maximum quantity of total renin, active renin comprised as little as 8% of the total renin (when plasma from a sodium-depleted subject was run) or as much as 40% of total renin (in eluates from sodium-depleted subjects). When the blank and active renin were subtracted from total renin, the resulting peak of inactive renin appeared in the same eluates as the peak of total renin, although the shape of the curve was changed by eliminating the skew of the curve of “total” renin caused by the presence of active renin in plasma of sodium-depleted subjects.
TABLE 1. Effect of Sodium Intake on Active and Inactive Renin in Plasma of Normal Men and Women

<table>
<thead>
<tr>
<th>Sodium intake</th>
<th>Urine sodium (mEq/day)</th>
<th>Active renin (ng/ml/hr)</th>
<th>Inactive renin (ng/ml/hr)</th>
<th>Total renin (ng/ml/hr)</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>8 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
<td>37 ± 4</td>
<td>16</td>
</tr>
<tr>
<td>Normal</td>
<td>115 ± 18</td>
<td>9 ± 2</td>
<td>18 ± 2</td>
<td>26 ± 3</td>
<td>11</td>
</tr>
<tr>
<td>High</td>
<td>398 ± 40</td>
<td>2.3 ± 0.5</td>
<td>14 ± 2</td>
<td>17 ± 2</td>
<td>9</td>
</tr>
</tbody>
</table>

*Blood drawn at noon from ambulatory volunteers. In four men, blood was drawn at 8:00 a.m. before arising. On low-sodium diet, active renin was 9.5 ± 2, and inactive renin was 12 ± 5. On normal-salt diet, active renin was 2.5 ± 0.6, and inactive renin was 12 ± 4.

Results

Activation of Inactive Renin in Plasma

Under optimal conditions, previously established for activation of inactive renin, treatment of normal plasma with pepsin or trypsin yields the same quantity of total renin as produced by dialysis to pH 3.3 and 7.5 (fig. 1). Treatment of plasma by a second method, after full activation by acid dialysis or protease, does not result in further increase in renin activity.

Active and Inactive Renin in Normal Plasma: Effects of Sodium Intake

Measurements in normal plasma are presented in table 1. Active renin increases more than 8-fold when sodium intake is reduced from more than 300 mEq/day to less than 10 mEq/day. Total renin in acid-dialyzed plasma also increases as sodium intake is reduced. Inactive renin is much less affected by sodium intake, being slightly lower in the volunteers on very high sodium intake, but not consistently increased when normal sodium intake was reduced. Changing posture had little effect on inactive renin, while active renin was increased in the standing posture.

Gel Filtration of Plasma: Sephadex G-100

Figure 2 depicts the elution pattern of active renin in unmodified eluates, and of total renin in eluates activated by dialysis, trypsin, or pepsin. Plasma collected when the subject was taking a low-sodium diet is compared with another specimen drawn on a high sodium intake. In both specimens, active renin eluted with ovalbumin, indicating a molecular weight near 40,000 daltons. The amount of active renin was consistently greater when the subject was on a low-sodium diet. Very little activation occurred during dialysis (pH 3.3 → 7.5) of eluates, but when pepsin 50 µg/ml was added to each eluate at pH 3.3, a large peak of total renin was demonstrated in eluates near serum albumin and ovalbumin (apparent molecular weight 58,000). Recovery of inactive renin (total minus active) in eluates incubated with trypsin 0.1 mg/ml was consistently lower (mean 56%) than in eluates incubated with pepsin (mean 100%). The quantity of total renin activated by trypsin (0.1 mg/ml) was similar to that activated by pepsin in eluates near serum albumin, but fell progressively to a level less than that of active renin in eluates of lower molecular weight, suggesting that active renin is probably destroyed by this concentration of trypsin in the latter eluates. Plasma from three normal subjects was run on Sephadex G-100 with similar results.

Sephacryl S-200

Paired samples (on low-sodium diet and on normal or high sodium intake) were run on Sephacryl S-200. Active renin, eluting with ovalbumin, was found in higher concentration in plasma drawn when subjects were on the low-sodium diet (figs. 3–4). Dialysis of eluates (pH 3.3 → 7.5) resulted in small increases in renin activity.

When the eluates were activated by pepsin, a large peak of total renin of apparent molecular weight 53,000 appeared between albumin and ovalbumin.
**Figure 2.** Renin activity in eluates of plasma of normal man from Sephadex G-100. Left panel: Subject on low-sodium diet. Right panel: Same subject on high-sodium intake. Solid lines: optical density indicating peaks of human serum albumin (Kav 0.2) and ovalbumin (Kav 0.33). Solid squares: renin in eluates treated with pepsin. Open circles: renin in eluates incubated with trypsin. Triangles: renin in eluates dialyzed to pH 3.3 and back to pH 7.5. Solid circles: active renin in untreated eluates.

**Figure 3.** Renin activity in eluates from Sephacryl S-200. Same plasma of normal male on low- and high-sodium intake as in figure 2. Symbols for treatment of eluates same as in figure 2.
When active renin in each eluate was subtracted from total renin found in that eluate after pepsin treatment, the maximum pepsin-activated renin appeared in the same eluate as the peak of total renin. Recovery of total renin in pepsin-treated eluates averaged 94%. The elution profiles of active renin and of pepsin-activated, inactive renin were generally similar in the three men and two women whose plasma has been studied. However, when plasma from three men was placed on Sephacryl S-200 and the eluates were activated with trypsin (0.1 mg/ml), less than half of the total renin (activated by pepsin) was recovered. In the fractions eluting near ovalbumin, total renin found after incubation with trypsin (0.1 mg/ml) was less than active renin in untreated eluates. The apparent peak of trypsin-activated renin was near serum albumin, but in later eluates, less renin was activated by trypsin (0.1 mg/ml) than by pepsin (figs. 2-4).

A possible destructive effect of trypsin on active renin was investigated by adding the same quantity of partially purified human renal renin to a series of eluates from gel filtration of renin-suppressed plasma, and incubating an aliquot of each with trypsin (0.1 mg/ml). In eight fractions eluting with and following serum albumin, the recovery of added renin in trypsin-treated aliquots was 92% ± 2% of that in control tubes not incubated with trypsin. In six fractions spanning the ovalbumin peak, less than 80% ± 2% of the added renin was recovered after incubation with trypsin (0.1 mg/ml). Clearly, trypsin can destroy active renin, but...
the effect of a single concentration of trypsin varies in different eluates.

**Gel Filtration of Activated Renin**

When plasma was incubated with pepsin or trypsin, resulting in full activation of renin, and then run on Sephacryl S-200, the activated renin eluted between albumin and ovalbumin. The running rate of activated renin in protease-treated plasma was very similar to the main peak of inactive renin in untreated plasma (fig. 5), indicating that apparent molecular weight remained the same after activation by protease.

A small amount of faster-running renin activity eluted with serum albumin when untreated plasma was run on Sephacryl S-200 (but not on Sephadex G-100). When plasma was incubated with pepsin or trypsin before gel filtration, little or no faster-running renin was seen (fig. 5).

**Discussion**

The renin activity of normal plasma increased by 2- to 10-fold when the plasma was dialyzed to pH 3.3 and then to pH 7.5. The same increase in renin activity was observed when the plasma was incubated with pepsin at pH 3.3, or with optimal amounts of trypsin at pH 7.5. Combining two methods (dialysis plus pepsin, dialysis followed by trypsin, pepsin followed by trypsin) did not increase the yield of active renin. Evidently, all these methods convert the same quantity of inactive renin in plasma to an enzyme which resembles active renin in kinetics and immunologic reactions.

The amount of inactive renin in normal plasma was not affected by dietary sodium to the same extent as active renin. Active renin progressively increased as dietary sodium was reduced. The proportion of total renin as inactive renin was highest (85%) in subjects on a high sodium intake; lower (66%) when dietary sodium was normal; and least when sodium intake was very low. Active renin increased after standing, but we observed no consistent change in inactive renin with upright posture. Similar changes have been described by other investigators in response to alterations in dietary sodium or posture.

Infusion of furosemide or diazoxide can increase both active and inactive renin in plasma, while propranolol may decrease active renin without similar effect on inactive renin.

In earlier studies, dialysis to pH 3.3 - 7.5 readily demonstrated the large amounts of big renin in pathological plasma, although only 10-15% of the total renin present was activated in eluates after gel filtration. In plasma of normal subjects on a low-sodium diet, containing large amounts of active renin and relatively little inactive renin, dialysis (pH 3.3 - 7.5) of eluates did not consistently detect the presence of big renin, which can be readily demonstrated after eluates have been incubated with pepsin.

Inactive renin of normal plasma has an apparent molecular weight (MW) of 58,000 on Sephadex G-100 and 53,000 on Sephacryl S-200 as demonstrated by pepsin activation of eluates from two different gels. Both estimates are significantly larger than active plasma renin, which elutes with ovalbumin, indicating a molecular weight near 41,000. The small difference in running rate of inactive renin on the two gels may be due to differences in charge or to anomalous behavior of glycoproteins on gel filtration. Inactive renin in plasma of patients with diabetic nephropathy is also activated by pepsin and closely resembles the inactive renin of normal plasma in its molecular weight and other properties. Atlas et al. and Yokosawa et al. have proposed very similar estimates of 58,000 and 56,000 for the MW of inactive renin in human plasma. Thus, most observers now agree that big renin exists in normal human plasma, as well as in the plasma of patients with diseases such as diabetic nephropathy or renin-secreting Wilms tumor.

The kidney is a probable source of inactive renin in plasma, although not the only source. Day et al. described big renin in a renal tumor. Slater and Haber extracted an inactive renin of MW near 60,000 from neutral homogenates of human kidneys, protected from proteolytic activity by a mixture of potent protease inhibitors. Following nephrectomy, both active and inactive renin decline precipitously, but inactive renin does not disappear from plasma. Non-renal sources of inactive renin include salivary glands and placenta and amniotic fluid.

Trypsin is as effective as pepsin in activating plasma renin, and is equally effective in diluted plasma provided that a lower concentration of trypsin is used. However, a single concentration of trypsin (0.1 mg/ml), selected for optimal yield in diluted plasma, was not uniformly successful in activating renin in eluates from gel filtration. In fractions eluting near serum albumin, the yield of activated renin was equal to that obtained with pepsin, but trypsin-activated renin declined relative to pepsin-activated renin in later eluates. Trypsin can destroy renin added to eluates. This destructive effect of trypsin is probably responsible for the fall of renin in trypsin-treated eluates to levels below the endogenous active renin.

Atlas et al. found that a low concentration of trypsin released more active renin in slower-running fractions, while more trypsin gave better yields in earlier fractions containing a trypsin inhibitor. The different levels of trypsin, required to activate renin fully and to avoid destroying active renin in various eluates, complicate the use of trypsin.

Activation of big renin in vitro can occur without a demonstrable change in molecular weight. Antibody, raised by injecting purified human renal renin into animals, reacts with and inhibits the enzymatic action of both the (endogenous) active renin of plasma and the activated form of inactive plasma renin, suggesting a similar structure near the active site. Trypsin and pepsin alter the structure of inactive renin to make its active site available, possibly by cleaving a small peptide fragment or changing the tertiary structure. Since pepsin and trypsin have different
specificities for peptide bonds, conceivably the active renin produced by each is slightly different. In the concentrations used, trypsin, but not pepsin, destroys active renin. This finding suggests that a bond specific for trypsin, adjacent to lysine or arginine, is probably located at or near the active site of renin. Inagami et al.* suggested that the beta-carboxyl of aspartic acid occurs near the active site of renin. Further characterization of the active renin produced by the action of different proteases upon inactive renin will help to define the chemical and biological relations between various precursors and enzymes resembling renin.

References

Inactive renin of high molecular weight (big renin) in normal human plasma. Activation by pepsin, trypsin, or dialysis to pH 3.3 and 7.5.

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