An inactive, prorenin-like substance in human kidney and plasma

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Summary

1. Plasma prorenin (inactive renin), which accounts for about 70% of the total renin in human plasma, was almost completely separated from active renin by affinity chromatography on Cibacron blue F3G-A-agarose. The slight residual renin activity present in the prorenin peak can be removed on concanavalin A-Sepharose, demonstrating that prorenin is completely inactive.

2. The renin activity of both human renal cortical extract and renal perfusate increased after incubation with trypsin. This trypsin-activable renin accounted for 15 and 40% of the total renin in extract and perfusate respectively.

3. Trypsin-activable renin from both renal extract and renal perfusate was, like plasma prorenin, almost completely separated from active renin on Cibacron blue F3G-A-agarose. After additional chromatographic steps, the trypsin-activable renin from renal cortical extract was found to be completely inactive.

4. We conclude that human kidney contains, and is able to release, a trypsin-activable renin that resembles plasma prorenin. It may differ from many of the 60 000 molecular-weight forms of renin previously identified in renal extracts, since these possess considerable intrinsic renin activity and probably represent a complex of renin with a binding protein.

Key words: affinity chromatography, Cibacron blue F3G-A-agarose, kidney, prorenin, renin.

Introduction

Human plasma contains a prorenin-like substance (inactive renin) that can be detected as renin-like activity after limited proteolysis. 'Acid-activation' (Lumbers, 1971) and 'cryoactivation' (Sealey, Moon, Laragh & Alderman, 1976) of prorenin depend upon endogenous plasma cofactors. Activation during dialysis to pH 3-3 is only partial (Atlas, Sealey & Laragh, 1978a, b) and may involve an acid protease (Morris & Lumbers, 1972). Activation is mediated predominantly by neutral serine proteases, including Hageman factor-activated prekallikrein, after inactivation of protease inhibitors at acid pH or at low temperature (Atlas et al., 1978a, b; Derkx, Bouma, Schalekamp & Schalekamp, 1979; Sealey, Atlas, Laragh, Silverberg & Kaplan, 1979). Because of this cofactor dependence, reproducible activation in chromatographic fractions requires the use of exogenous proteases (Shulkes, Gibson & Skinner, 1978; Atlas, Sealey, Laragh & Warekois, 1979b; Sealey, Atlas & Laragh, 1980). With trypsin, the molecular weight of inactive plasma renin is approximately 57 000 (Atlas et al., 1979b; Yokosawa, Takahashi, Inagami & Page, 1979; Sealey et al., 1980). After partial purification by affinity chromatography, it has been shown to be devoid of intrinsic renin activity (Yokosawa et al., 1979; Atlas, Sealey & Laragh, 1979a).

Large-molecular-weight (60 000) renins have been identified in renal cortical extracts of several species (Boyd, 1974; Day, Luetscher & Gonzales, 1975; Leckie & McConnell, 1975; Inagami & Murakami, 1977; Levine, Lentz, Kahn, Dorer & Skeggs, 1978; Potter, McDonald, Dunn & Metcalfe, 1978; Slater & Haber, 1978). With the possible exception of that found in Wilms' tumour extracts (Day et al., 1975), the 60 000 molecular-weight kidney renins described to date appear to differ from inactive renin in human plasma in that all possess considerable enzymatic activity. Recent evidence suggests that many of these substances may result from the binding of active (40 000 molecular weight) renin to a cortical polypeptide during extraction (Funakawa, Funae
Since the inactive renin found ubiquitously in human plasma more closely resembles a potential renin precursor, we have attempted to isolate a similar substance from human kidney, focusing on the issue of intrinsic activity rather than of molecular size. We report herein on the existence of a pro-renin-like substance in both extracts and perfusates of normal human kidney.

**Methods**

Plasma (collected in EDTA), pooled from untreated hypertensive patients without renal disease, was dialysed overnight against 100 vol. of buffer 1: Tris-Cl (50 mmol/l), pH 8, containing NaCl (100 mmol/l), EDTA (6 mmol/l) and benzamidine/HCl (20 mmol/l). Renal cortical extract was prepared from a kidney of a patient with unilateral renal artery stenosis, which was frozen within 1 h of excision. Cortex (1-3 g) was dissected, minced and homogenized in 5 vol. of buffer 1. Immediately after two passes (45 s each) in a Teflon–glass homogenizer, di-isopropylfluorophosphate (DFP) and phenylmethylsulphonylfluoride (PMSF) were added (5.7 mmol/l each); the sample was homogenized once after each addition. The homogenate was then frozen and thawed three times, homogenized again, and centrifuged in a Beckman Spinco ultracentrifuge (100 000 g for 60 min). The supernatant was chromatographed immediately (see below). Renal perfusate was obtained by perfusing a normal human kidney (transplant donor) with Plasmanate (Cutter Laboratories) at 4-6°C, with a Waters pulsatile pump at 0.5-2 ml min⁻¹ g⁻¹ of kidney. The perfusing solution, which contained no angiotensinogen and very low renin activity (0.9 pmol of ANG I h⁻¹ ml⁻¹), was recirculated for 8 h, during which time renin activity progressively increased to 42 pmol of ANG I h⁻¹ ml⁻¹. Aliquots removed at 1, 2, 4 and 8 h were pooled and, after addition of EDTA, benzamidine, DFP and PMSF at the above concentrations, dialysed against buffer 1.

Affinity chromatography on Cibacron blue F3G-A–agarose (Affi-Gel Blue). Samples were applied to a 2.5 cm × 20 cm column of gel equilibrated with buffer 1. Flow rate was controlled by pump (Pharmacia) at 1.0 ml/min, and 10 min fractions were collected with an LKB Ultrorac II. After extensive washing with buffer 1, trypsin-activable renin was eluted with the same buffer containing 1.0 mol of NaCl/l (arrow). Fractions were assayed for active renin (○) and trypsin-activated total renin (●) as described in the Methods section. (a) Plasma (10 ml), which had active and total renin contents of 7.9 and 23.8 pmol of ANG I h⁻¹ ml⁻¹; recoveries were 90 and 86% respectively. (b) Renal perfusate (7.5 ml), which had active and total renin contents of 26 and 43 pmol of ANG I h⁻¹ ml⁻¹; recoveries were 92 and 80% respectively. (c) Renal cortical extract (5 ml), which had active and total renin contents of 4700 and 5400 pmol of ANG I h⁻¹ ml⁻¹; recoveries were 84 and 80% respectively.

Renin activity was measured as the rate of ANG I formation at 37°C, pH 5.7, in the presence of PMSF, EDTA and homologous angiotensinogen (500 pmol/ml) purified by modification (Atlas et al., 1978a) of the method of Tewksbury, Premeau & Dumas (1976).
Incubation times and sample dilution were chosen so that substrate utilization was less than 5%. Inactive renin was determined as the difference in activity between activated and unactivated samples (i.e. 'total' — active renin). Activation was performed in buffer I diluted 1:1 with Tris–Cl (50 mmol/l), pH 8, containing 1% bovine serum albumin (10 mmol/l, final benzamidine concentration); samples were incubated for 1 h at 25°C after adding bovine pancreatic trypsin (100 µg/ml), and the reaction was stopped by adding soyabean trypsin inhibitor (100 µg/ml) and PMSF (5.7 mmol/l) and adjusting to pH 5.7 with maleic acid (0.27 mol/l), 40 µl/ml. Active renin was measured under identical conditions, except that trypsin was omitted. The ANG I formed was quantified by radioimmunoassay (Sealey, Gerten-Banes & Laragh, 1972).

**Results**

The majority of active plasma renin was not retained by Affi-Gel Blue (Fig. 1a), as previously reported (Carlson, Hsueh & Luetscher, 1978; Atlas et al., 1979a; Johnson, Poisner & Crist, 1979; Yokosawa et al., 1979). Inactive renin (which comprised 70% of the total renin) was bound and could be eluted with increasing ionic strength (Fig. 1a). Recoveries of active and inactive renins were 90 and 85% respectively. A small amount of renin activity appeared near the inactive renin peak but was less than 5% of the total activity. This residual active renin can be removed by affinity chromatography on pepstatin-Sepharose (Yokosawa et al., 1979) or concanavalin A-Sepharose (Atlas et al., 1979a).

A similar pattern was obtained when renal perfusate or renal cortical extract was applied to the same column (Fig. 1b and 1c). The perfusate had a renin activity of 26 pmol of ANG I h⁻¹ ml⁻¹, which increased to 43 pmol of ANG I h⁻¹ ml⁻¹ after incubation with trypsin. Renal extract had a much greater renin activity (4700 pmol of ANG I h⁻¹ ml⁻¹), which increased by only 15% with trypsin. With both perfusate and extract, the trypsin-activatable form was selectively retained by Affi-Gel Blue. As with plasma, a small amount of renin activity appeared near the trypsin-activatable peaks from perfusate and extract, but this constituted less than 5% of the total activity in the later-eluting fractions (Fig. 1b and 1c).

The fractions containing trypsin-activatable renin from renal cortex were pooled and applied to a column of pepstatin–aminohexyl–Sepharose. The residual active renin was bound to the affinity gel, but the trypsin-activatable renin was not adsorbed and was thus found to be completely inactive.

**Discussion**

In this report we have described for the first time the existence in normal human kidney of an activatable form of renin that, like inactive plasma renin, is bound by Cibacron blue–agarose (Affi-Gel Blue). Additional chromatographic steps have revealed that the prorenin-like substance in plasma is completely inactive (Atlas et al., 1979a; Yokosawa et al., 1979). This is true, as well, of the trypsin-activatable renin isolated from human renal cortex, since it is devoid of activity with homologous substrate after passage over pepstatin-Sepharose. Our preliminary data suggest that it has an apparent molecular weight in the 50 000–55 000 range, which falls to approximately 45 000 after trypsin activation. This inactive renin represents only 15% of the total renin extracted from renal cortex, whereas in plasma it is the major form, accounting for as much as 90% of the total renin in normal human subjects (Sealey et al., 1980). We have found an intermediate proportion (about 40%) in kidney perfusates. If these substances are the same, this observation suggests that circulating inactive renin may have a longer biological half-life than active renin and/or that it is rapidly degraded after extraction from renal tissue.

In contrast to this trypsin-activatable inactive renin, the large-molecular-weight (60 000) renins previously isolated from mammalian kidneys have considerable intrinsic activity and often constitute a larger proportion of renal renin. When extraction is performed in the presence of thiol protease inhibitors, renin may be exclusively in the 60 000 molecular-weight form, leading to speculation that this may be the native form of kidney renin (Inagami, Hirose, Murakami & Matoba, 1977; Slater & Haber, 1978; Potter, Dunn & McDonald, 1979). However, Funakawa et al. (1978) have found that sodium thiobionate and N-ethylmaleimide probably act not by inhibiting thiol proteases but rather by promoting disulphide bond formation between 40 000 molecular-weight renin and an acid-labile polypeptide in renal cortex; their data suggest that formation of large-molecular-weight renal renin may be a reversible, post-translational event and that its existence may even possibly be artifactual.

It has often been assumed that the 60 000 molecular-weight renins in animal kidneys are
analogous to inactive renin in human plasma, principally because they have similar apparent molecular weights and are both activated by acidification. But these similarities may be coincidental. The most striking difference between these substances is that the 60 000 renal renins are all largely active. They have been identified, in fact, by virtue of their size rather than by their lack of activity. Conversion to 40 000 molecular-weight renin has been reported to occur 'spontaneously' (Levine et al., 1978; Potter et al., 1979) or after acidification (Boyd, 1974; Leckie & McConnell, 1975; Potter et al., 1978), but this is never associated with more than a two- to three-fold increase in activity (Inagami et al., 1978). Acidification actually decreased the total activity of 60 000 molecular-weight hog kidney renin, and we have observed a loss in activity of human renal extracts after acidification (unpublished work). Others have reported that trypsin (Potter et al., 1978) or thrombin (Overturf, Druilhet & Fitz, 1979) can increase the activity of large-molecular-weight active renins from dog or hog kidney, but again the increases were small (only one- to four-fold).

Although the trypsin-induced increase in renin activity of crude renal cortical extract that we have observed is also rather small, this increase is clearly attributable to a distinct molecular species which can be separated chromatographically from active renin and shown to be completely inactive. The strength of these preliminary observations rests with the apparent similarity of this substance to inactive plasma renin, whose characterization does not require complex extraction procedures that risk artifacts. The resemblance of the inactive renin in renal cortex to that found in plasma is amplified by our finding that a similar substance is released by the perfused human kidney. Although further characterization is required before it can be classified as a precursor of renin, it is likely to be a substance of physiological relevance.

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References


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