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# Identification of an enzyme in human kidney that correctly processes prorenin

(renin/processing/proenzyme/secretory granule)

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ABSTRACT **Using pure recombinant human prorenin as a substrate, we have identified an enzyme in human kidney that accurately processes prorenin to active renin (EC 3.4.23.15). In the crude homogenate, the predominant activity of this potential renin-processing enzyme (RPE) converted the $M_r$ 47,000 inactive prorenin to $M_r$ 44,000 active renin and had a pH optimum of ≈6. The activity was blocked by cysteine protease inhibitors, but not by pepstatin, EDTA, or serine protease inhibitors. This RPE activity was not detected in a similarly prepared homogenate of human chorion decidua tissue, which produces primarily prorenin, or in human plasma. The activity was purified 100-fold by ammonium sulfate precipitation, *p*-chloromercuribenzoate affinity chromatography, and chromatofocusing. The partially purified enzyme has a $M_r$ of ≈27,000 and an isoelectric point in the pH 4.8–5.6 range. The activity in the purified RPE preparation had the same pH optimum as that in crude homogenate, cleaved the prosegment at the same site used by the kidney *in vivo* based on amino-terminal sequencing of the processed renin, and did not degrade prorenin or renin. These data suggest that the cysteine protease we have isolated is a candidate for authentic renal RPE.**

Generation of renin (EC 3.4.23.15) from its inactive biosynthetic precursor, prorenin, may play a crucial role in the production of the active form of this enzyme by the kidney (1, 2). Renin regulates the production of angiotensin I and, hence, angiotensin II, which is a major determinant of blood pressure and intravascular volume (3). Its availability determines overall activity of the system. The conversion of prorenin to renin may be of regulatory importance, since the relative plasma concentrations of prorenin and renin vary under different conditions (1, 2), and in diabetic nephropathy prorenin may be released without substantial release of renin (4). The generation of renin from prorenin involves partial proteolytic cleavage of the 43-amino acid prosegment after the dibasic residues, Lys-Arg, leaving leucine as the first amino acid in renin (5, 6). This processing step probably occurs in the storage granules of the renal juxtaglomerular cells, since prorenin is present in immature granules and disappears as the granules mature (7). Thus, physicochemical properties of the renin-processing enzyme include: (*i*) localization in the juxtaglomerular cells, (*ii*) activation of prorenin, and (*iii*) cleavage of the 43-amino acid prosegment without further destruction of active renin.

Several enzymes have been demonstrated to activate human prorenin (reviewed in refs. 1 and 8). These include trypsin, plasmin, plasma and tissue kallikrein, epidermal growth factor, pepsin, and cathepsin B. However, the data available to date do not establish whether any of these enzymes is the authentic renal prorenin-processing enzyme. Of those examined, only trypsin has been shown to cleave prorenin at the same site used by the kidney (9, 10). However, trypsin also cleaves prorenin at other sites, will degrade renin, and has not been found in human kidney (1). Kallikrein has been found in high concentrations in the cells of the vascular pole of the glomerulus but not in the juxtaglomerular cells (11). Cathepsin B, ordinarily a lysosomal enzyme (12), has been found in the juxtaglomerular cells (13); by methods similar to those used for purifying hepatic cathepsin B, Slater *et al.* (14) purified a cathepsin B-like enzyme from human kidney that activated plasma prorenin, although the site of cleavage was not determined.

In this study, we started with crude homogenate of human kidney to characterize and partially purify the predominant enzyme that activates prorenin. The availability of recombinant prorenin as a substrate has allowed us to avoid potential problems of contaminating plasma proteases and has provided enough material to determine the precise site of cleavage. The results indicate that the predominant processing activity is a thiol protease, that it activates prorenin by cleaving the same site clipped in native renin, and that it neither cleaves prorenin at other sites nor degrades the mature enzyme.

## METHODS

**Purification of Recombinant Prorenin.** Recombinant prorenin was obtained from cultured Chinese hamster ovary (CHO) cells transfected with a vector that expresses the human renin gene (15). Medium (40 L) from these cells was concentrated by a Pellicon concentrator (Millipore, model 42 00K 50) to 1 liter equilibrated against 20 mM sodium phosphate (pH 7.0) containing the protease inhibitors EDTA (10 mM), phenylmethylsulfonyl fluoride (PMSF, 2 mM), 8-hydroxyquinoline (1 mM), aprotinin (20 kallikrein inhibitor units/liter), and benzamidine (20 mM). All purification steps were at 4°C. The concentrated medium (280 ml) was adjusted to 1 M NaCl and applied to a phenyl Sepharose column (2.5 × 10 cm) (Pharmacia). The column was washed with 3 column vol of the same buffer and prorenin eluted with 25% (vol/vol) ethylene glycol in water. The prorenin-containing fractions were pooled (38 ml), dialyzed against 20 mM sodium acetate (pH 6.0) at 4°C for 24 hr, and applied to a pepstatin Sepharose affinity column (2.5 × 6 cm), which removed active renin (6). The pepstatin pass-through fractions were combined (110 ml) and applied to cibacron blue affinity column (1.5 × 10 cm) (ref. 16; Bio-Rad). The bound prorenin eluted with a NaCl gradient (0–1 M) in 25 mM sodium phosphate buffer (pH 7.0). The eluted fractions (23 ml) were

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Abbreviations: PMSF, phenylmethylsulfonyl fluoride; RPE, renin-processing enzyme; GU, Goldblatt unit(s).

Table 1. Purification of recombinant prorenin

| | Prorenin, GU | Protein, mg | SA, GU/mg protein | -fold increase in SA | Yield, % |
|---|---|---|---|---|---|
| Medium concentration | 1355 | 235 | 5.7 | — | — |
| Ammonium sulfate | 1030 | 150 | 6.8 | 1.2 | 51 |
| Phenyl Sepharose | 894 | 61 | 14.6 | 2.6 | 44 |
| Pepstatin (pass-through) | 455 | 22 | 20.7 | 3.6 | 30 |
| Affi-Gel Blue | 285 | 0.38 | 950 | 167 | 23 |
| HPLC | 178 | 0.17 | 980 | 172 | 18 |

See *Methods* for details. SA, specific activity expressed for prorenin concentration.

concentrated to 1 ml on a Centricon-10 (Amicon) concentrator and subjected to gel filtration HPLC on a 30-cm TSK-3000 (Bio-Rad) preparative column (16).

**Isolation and Assay of Renin-Processing Enzyme (RPE) Activity.** Human renal cortex (10 g) was obtained from cadaver kidneys, frozen, and stored at −20°C, homogenized by Polytron (Brinkmann) in 25 mM sodium phosphate buffer (pH 6.0) at 4°C (1 g of tissue per 2 ml of buffer), and centrifuged at 10,000 rpm for 20 min at 4°C. Twenty milliliters of the supernate (28 mg of protein per ml) was applied to a pepstatin affinity column (1.5 × 6 cm) to remove endogenous active renin [$<10^{-5}$ Goldblatt units (GU)/ml]. Pepstatin pass-through fractions (50 μl; 25 mg of protein per ml) were then incubated in the same buffer at 37°C for 2 hr with pure recombinant human prorenin (1–3 GU) prepared as described above.

**Measurement of Active Renin Generated by RPE in Kidney Homogenates.** The active renin concentration in the incubate was measured after radioimmunoassay of angiotensin I (AI) generated from sheep angiotensinogen (6); in this assay, 120,000 ng of $AI \cdot ml^{-1} \cdot hr^{-1}$ represents 1 GU of renin as determined against renal renin (68–356 GU) from the National Institute of Biological Standards and Controls (Holly Hill, London). The total renin concentration (prorenin plus renin) was determined by measurement of the active renin concentration after treatment with optimal concentrations of trypsin (1). In the enzyme assay, the prorenin concentration of the kidney preparation (usually <10 mGU/ml) is subtracted from the preparation incubated with recombinant prorenin.

**Determination of Prorenin Cleavage by RPE.** Recombinant prorenin (1–3 GU) was incubated with pepstatin-treated renal cortical homogenate (50 μl). Ten microliters of this mixture was subjected to immunoblot analysis developed with an antibody against pure human renal renin, which cross-reacts with both renin and prorenin (17). In these assays, endogenous renin is undetectable (1 ng of renin is required) and endogenous prorenin is barely detectable and contributes negligibly to the total prorenin. Conversion of $M_r$ 47,000 prorenin to $M_r$ 44,000 renin indicated the presence of RPE activity.

**Characterization of the RPE.** The pH optimum of activity was determined by immunoblot analysis of recombinant prorenin incubated with pepstatin-treated kidney homogenate at pH 4.0–8.0 for 2 and 24 hr at 37°C. The effects of protease inhibitors to inhibit RPE activity were tested at the pH optimum for 2 hr at 37°C. Inhibitors included the metalloprotease inhibitor EDTA (10 mM); the aspartyl protease inhibitor pepstatin (1 mM); serine protease inhibitors benzamidine (20 mM), PMSF (0.2 mM), aprotinin (0.1 ng/ml), and soybean trypsin inhibitor (0.1 mg/ml); and thiol protease inhibitors leupeptin (1 mM), E-64 (0.1 mM), *p*-hydroxymercuribenzoic acid (0.1 mM), 2,2-dithiodipyridine (1 mM), and iodoacetic acid (1 mM). The isoelectric point of RPE was assessed by activity in fractions following chromatofocusing (Pharmacia) of pepstatin-treated kidney homogenate. The molecular weight was estimated by gel-filtration HPLC.

The site of RPE cleavage of recombinant prorenin was determined by amino-terminal sequencing of pure renin obtained after incubation of semipurified RPE with pure recombinant prorenin at 37°C and pH 6.0 for 24 hr. RPE was semipurified as described in *Results*, once the enzyme inhibitor profile of renin-processing activity was identified. Active processed renin was purified from the incubate by affinity chromatography with H-77, a synthetic octapeptide renin inhibitor (6, 18). Amino acid sequencing was performed on the Applied Biosystems model 370A gas-phase microsequencer (19). The phenylthiohydantoin (PTH) derivative was analyzed by the on-line PTH analyzer (Applied Biosystems model 120).

## RESULTS

**Purification of Recombinant Prorenin.** A scheme for the purification of human recombinant prorenin is given in Table 1. Nearly half of the total renin in the concentrated CHO medium was active; this was removed by pepstatin affinity chromatography (6). Renin in the pepstatin pass-through fractions was 97% inactive at 0°C or 37°C and remained as such during subsequent steps. The prorenin bound to Affi-Gel Blue and eluted with 0.5 M NaCl (16). Prorenin eluted from HPLC with a single band of $M_r$ 47,000, characteristic of recombinant prorenin (16). The purification resulted in 170 μg of prorenin with a specific activity of 980 GU per mg of protein and an overall yield of 18%. SDS gel electrophoresis demonstrated a major protein band with a molecular weight the same as that determined by HPLC (Fig. 1). This band shows some broadening, perhaps because of variations in glycosylation (9, 15). However, a band corresponding to active renin is not detectable. Thus, enzyme activity of the purified prorenin is primarily due to that of intact prorenin. The first 11 amino acids of pure recombinant prorenin were sequenced and were identical to those predicted by the cDNA sequence (20–23). Thus, the intact 43-amino acid prosegment was present in the purified prorenin.

**Characterization of Renin Processing Activity.** RPE activity in renal cortical homogenate passed through a pepstatin column was maximal at pH 4.0–6.0 (Fig. 2*A*) with some activity at pH 7.0, which markedly decreased at pH 8.0. The conversion at pH 6.0 was found to be time dependent with nearly complete conversion by 2 hr (data not shown). With

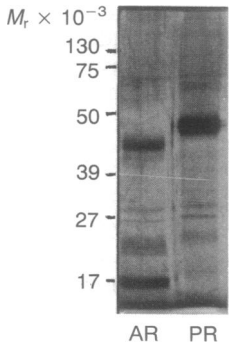



FIG. 1. Pure human renal renin (6) and pure recombinant prorenin run on SDS/PAGE stained with silver nitrate. Recombinant prorenin has a $M_r$ of 47,000, while renal renin has a $M_r$ of 44,000 with $M_r$ 22,000 and 18,000 fragments. AR, active renin; PR, prorenin.

prolonged incubation overnight there was destruction of prorenin and renin at pH 4.0 and 5.0 but no significant destruction at pH 6.0. The time course of generation of renin activity is shown in Fig. 2*B*. At 0 hr, 17% of the prorenin was active, probably due to activation while sitting with pepstatin-treated kidney homogenate in a 4°C ice bath for the duration of the experiment. With time of incubation at pH 4.0–7.0, there was increasing generation of enzymatically active renin, while negligible generation occurred at pH 8.0. The maximal rate of generation appeared to occur at pH 6.0 with 85% of the recombinant prorenin activated by 2 hr. When incubated alone at pH 4.0–8.0, 37°C pure recombinant prorenin levels remained stable at 21 GU as determined by trypsin activation with no change in its molecular weight by immunoblot analysis. That the generation of active renin was dependent on the RPE in the homogenate was also indicated by the finding that dilution of the preparation led to progressive loss of RPE activity (data not shown). Activity was nearly abolished when the extract was diluted 1:16. In addition, when recombinant prorenin was incubated with a homogenate of human chorion decidua prepared under the same conditions as kidney cortex or normal human plasma, at pH 6.0 and 37°C for 2 hr, no processing of prorenin was detected (data not shown).

The enzyme class of renin processing activity was determined by the effectiveness of enzyme inhibitors as followed by immunoblot analysis (Table 2). No inhibition of prorenin processing was seen with EDTA, pepstatin, or a variety of serine protease inhibitors. In contrast, the cysteine and serine protease inhibitor, leupeptin, was found to exhibit dose-dependent inhibition of processing that was complete at 1 mM leupeptin (Fig. 3). Leupeptin also inhibited generation of renin enzyme activity from inactive prorenin but did not interfere in the renin assay. Other cysteine protease inhibitors, E-64, *p*-hydroxymercuribenzoic acid, 2,2-dithiodipyridine, and iododiacetic acid were also effective inhibitors of RPE activity. The finding that RPE activity in the crude homogenate was completely blocked by the thiol protease inhibitors suggests that almost all of the extractable RPE in the kidney at pH 6.0 is of this class of enzyme.

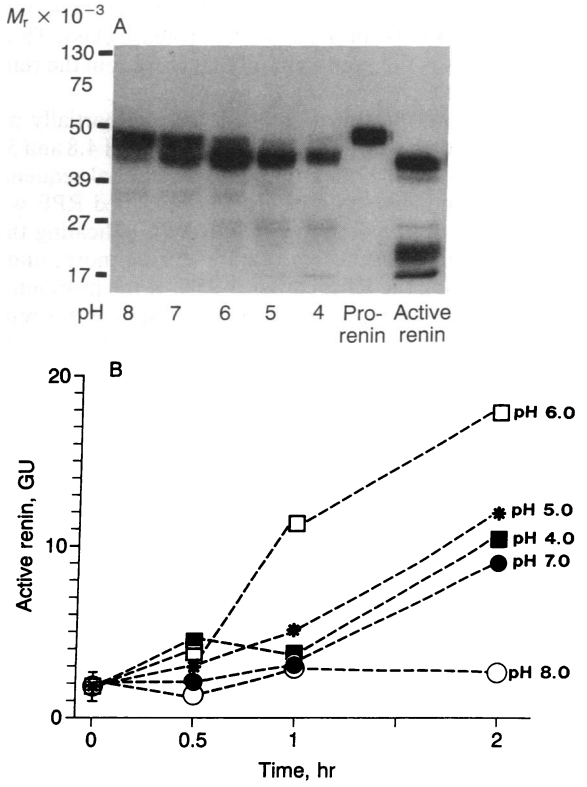



FIG. 2. (*A*) Immunoblot analysis demonstrating effect of pH on the ability of RPE to convert $M_r$ 47,000 recombinant prorenin to $M_r$ 44,000 renin. The reaction was performed for 2 hr at 37°C. (*B*) Effect of pH and time on generation of active renin from inactive recombinant prorenin at 37°C by pepstatin-treated kidney homogenate passed through pepstatin. Each point represents three measurements performed in duplicate. The total renin determined by trypsin treatment was 21 GU.

Table 2. Effects of inhibitors of the renin-processing enzyme

| | Concentration | Type of protease inhibitor |
|---|---|---|
| No inhibition | | |
| Benzamidine | 20 mM | Serine |
| EDTA | 10 mM | Metallo |
| Pepstatin | 1 mM | Aspartyl |
| PMSF | 0.2 mM | Serine |
| Aprotinin | 0.1 mg/ml | Serine |
| Soybean trypsin inhibitor | 0.1 mg/ml | Serine |
| Inhibition | | |
| Leupeptin | 1 mM | Thiol |
| E-64 | 0.1 mM | Thiol |
| *p*-Hydroxymercuribenzoic acid | 0.1 mM | Thiol |
| 2,2-Dithiodipyridine | 1 mM | Thiol |
| Iododiacetic acid | 1 mM | Thiol |

Inhibitors were tested at the indicated concentration during incubation of crude renal cortical homogenate (see text for details of preparation) with recombinant prorenin at pH 6.0 and 37°C for 2 hr.

**Partial Purification of the RPE.** The putative RPE was partially purified by homogenizing human renal cortex (730 g) in 20 mM sodium phosphate buffer (1500 ml) (pH 6.0) containing 1 mM EDTA. All procedures were performed at 4°C. After ammonium sulfate precipitation, the 25–65% fractions were dissolved in 50 mM sodium acetate (150 ml) (pH 6.0) containing 1 mM EDTA, dialyzed overnight against the same buffer, and applied to a *p*-chloromercuribenzoate agarose affinity column (2.5 × 12.0 cm) (Pierce). The column was washed with 3 column vol of 50 mM sodium acetate buffer (pH 6.0) containing 1 mM EDTA and 0.5 M NaCl and the bound RPE eluted with 20 mM cysteine in the same buffer. The eluted fractions were combined (70 ml total); dialyzed against 4 liters of 25 mM histidine hydrochloride (pH 6.0) containing 1 mM EDTA; and applied to a chromatofocusing column (1.5 × 10.0 cm) (PBE-94, Pharmacia), which was eluted with a pH gradient 6.6–4.2 (Polybuffer 74-HCl, Pharmacia). RPE activity was detected in fractions spanning pH 4.8–5.6 with major peaks at 5.0 and 5.3. The combined fractions contained 48 mg of protein in 60 ml of buffer. One milliliter of this preparation generated 2.5 GU of renin from 3.0 GU of prorenin, whereas 1 ml of kidney homogenate generated 0.7 GU of renin from the same amount of prorenin. This suggests approximately a 100-fold purification of the processing enzyme. This RPE preparation was used for determination of molecular weight and for preparation of processed renin for amino-terminal sequencing. Incubation

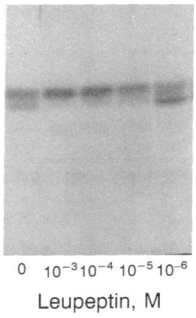



FIG. 3. Leupeptin inhibition of prorenin processing by pepstatin-treated kidney homogenate. No inhibition occurs with 10 mM leupeptin.

of the semipurified preparation of RPE with prorenin was generally performed with 5 mM cysteine. It eluted as a single peak with an estimated $M_r$ of 27,000 following gel filtration HPLC (Fig. 4).

To determine whether RPE destroys active renin, semipurified RPE (50 μl; 0.8 μg of protein per ml) was incubated with 5 μl (30 μg/ml) of pure active renal renin at pH 6.0 and 37°C for 2 hr. Although prorenin was converted to active renin under these conditions, neither this renin nor renal renin was further degraded to lower molecular weight forms as judged by immunoblot analysis (Fig. 5). In addition, renin concentration of the pure renal renin preparation was 150 mGU before and 140 mGU after incubation with RPE.

**Amino-Terminal Sequencing of the Processed Renin.** Six hundred micrograms of pure recombinant prorenin was obtained from several purifications and incubated overnight at pH 6.0 and 37°C with a semipurified RPE preparation containing ≈550 μg of protein in a final vol of 13 ml. Ninety-five percent of the prorenin was activated. Purification of the processed renin by H-77 affinity chromatography resulted in 360 μg of protein demonstrating a triplet on SDS/PAGE (Fig. 5, lane A) with a $M_r$ of ≈44,000 and a specific activity of 1200 GU per mg of protein. This represents an overall recovery of 63% of the processed renin, consistent with previous results for renal renin (6). The heterogeneity of processed renin is likely due to variations in glycosylation (9, 15), since microsequencing of this material indicated a single amino terminus, shown in Fig. 6*B*. Amino acid 5, a known glycosylation site, was not identified, since our microsequencing method does not consistently detect glycosylated amino acids. Thus, RPE clips the complete 43-amino acid prosegment from the amino terminus of prorenin after paired basic residues Lys-Arg.

## DISCUSSION

The availability of human recombinant prorenin is a key development to study the putative RPE. Using a five-step procedure, we purified human recombinant prorenin. The specific activity of the trypsin-treated material was 980 GU per mg of protein, which is similar to that reported for human active renal renin (6, 24–26) and is in general agreement with that reported for semipurified and pure preparations of recombinant prorenin (9, 27, 28). Pure prorenin was ≈97% inactive and the residual renin activity appeared to be due to prorenin rather than contaminating renin based on SDS/PAGE analysis of the preparation. This activity of prorenin may result from an equilibrium between inactive and active prorenin (10), since amino-terminal sequencing indicated that the 43-amino acid prosegment of purified prorenin was intact. Therefore, pure recombinant prorenin resembles the native substrate seen by RPE in terms of its amino acid sequence and low enzyme activity.

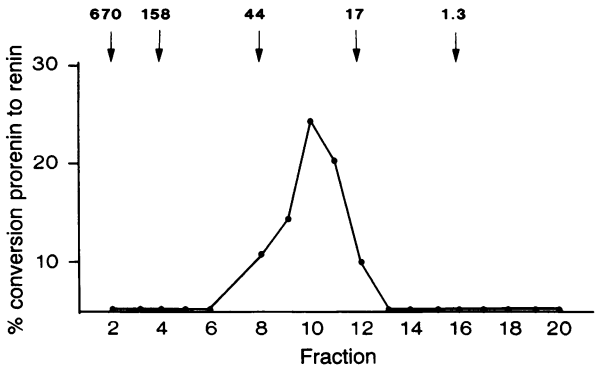



FIG. 4. Gel filtration HPLC of semipurified RPE. Arrows denote molecular weight markers ($\times 10^{-3}$). The molecular weight estimate of RPE is 27,000.

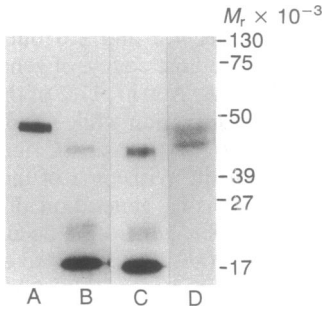
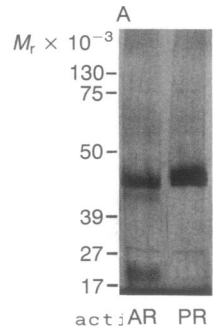



FIG. 5. Pure recombinant prorenin and pure human renal renin were incubated without (lane A, prorenin; lane B, active renin) and with (lane C, active renin; lane D, prorenin) semipurified RPE (see text) at 37°C and pH 6.0 for 2 hr. Although prorenin was processed to active renin (lane D), no change or destruction of native active renin occurred (lane B is not changed from lane C). The $M_r$ 22,000 and 18,000 subunits have been described (6).

Using recombinant prorenin as a substrate, we assayed homogenates of human renal cortex for RPE activity identified by conversion of $M_r$ 47,000 prorenin to $M_r$ 44,000 active renin. In crude homogenates, the pH optimum for the RPE was between 4 and 6. At pH 6.0 there was negligible destruction of the active renin generated, although the possibility of contaminating proteases was suggested by the finding that at pH 4.0 and 5.0 there was some destruction of renin with prolonged incubation. Cleavage and activation of prorenin by RPE is time and concentration dependent. Inhibitor studies indicate that essentially all of the RPE present in the kidney homogenate at pH 6.0 is of the cysteine protease class. Thus, this enzyme class is the predominant one present in the renal cortex that can cleave prorenin to renin.

The RPE was further purified 100-fold. The partially purified enzyme had an isoelectric point between pH 4.8 and 5.6 and an estimated $M_r$ of 27,000. The amino-terminal sequence of the active renin generated by partially purified RPE was identical to that of native active renal renin, indicating that this RPE correctly processes prorenin. Furthermore, under conditions in which the RPE converts 95% of the prorenin to active renin, there was no detectable conversion of this renin or of pure human renal renin to lower molecular weight degraded forms, suggesting that this activity resembles that *in vivo*.




B

| | 1 | | | | | | | | 10 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AR: | Leu | Thr | Leu | Gly | X | X | X | Ser | Ser | Val | Ile | Leu | Thr | Asn | Tyr | Met | Asp | Thr | Gln |
| PR: | Leu | Thr | Leu | Gly | X | Thr | Thr | Ser | Ser | Val | Ile | | | | | | | | |

FIG. 6. (*A*) SDS/PAGE of pure active human renal renin (AR) and pure recombinant prorenin treated with RPE (processed renin, PR). Both have a $M_r$ of 44,000. (*B*) Amino-terminal sequence of PR compared to that of AR (7).

The renal juxtaglomerular cells are the major source of circulating active renin, since nephrectomy results in an acute and profound drop in the plasma active renin concentration (29). Whether any of the extrarenal sites of prorenin production (30) process prorenin to renin has not been established. However, renin has been localized to secretory granules in the rat adrenal and in human pituitary (31). As discussed earlier, the secretory granule is likely the intracellular site of prorenin to renin processing. The intracellular pH of the renin secretory granules is unknown. In the pancreas, the insulin secretory vesicles have a pH of 5.0–6.0 with proinsulin to insulin conversion occurring at a greater rate in the more acidic vesicles (32). In our studies, RPE activity was optimal in the pH 4–6 range with greatly decreased activity at pH 8. In contrast, chorion decidua from normal pregnant women produces primarily prorenin, rather than active renin, which is secreted constitutively by membrane-bound vesicles (33). The finding that no RPE activity could be detected in plasma or in homogenate of human chorion decidua, a tissue that primarily produces prorenin, handled in an identical manner as renal cortical homogenate could imply that RPE is specific for certain tissues and that processing of prorenin is not the result of nonspecific tissue proteases in general for all tissues.

It is not possible to know whether any of the other enzymes that have been demonstrated to activate inactive prorenin *in vitro* (1, 8) are identical to the RPE we have isolated. These previous studies used impure preparations of human prorenin, which made it difficult to determine the exact site of the cleavage. In fact, prorenin can be activated by cleavage at other sites. For example, we have found that after exposure to pH 3.0 recombinant prorenin in CHO cell culture medium can be cleaved to an intermediate form of fully active renin with 11 amino acids of the prosegment still present (34). Heinrickson *et al.* (10) also reported the existence of active forms of renin containing prosegment sequences. Thus, the cleavage site specificity is an important requirement for the identification of the renal RPE. Furthermore, our studies with the protease inhibitors, PMSF and benzamidine, indicate that RPE is not a serine protease and, with aprotinin, suggest that RPE is also not kallikrein.

The thiol protease inhibitors leupeptin, *p*-chloromercuribenzoate, E-64, iodoacetate, and 2,2-dipyridyl sulfate are potent inhibitors of the RPE we have isolated. This inhibitor profile is similar to that of the lysosomal cysteine proteases cathepsins B, H, and L (12). The RPE we identified differs slightly from cathepsin B in that EDTA did not inhibit its activity (35). Previous studies suggested that a renal thiol protease and cathepsins B, H, and L can convert higher molecular weight prorenin to a lower molecular weight and activate human plasma prorenin (14, 36, 37). The kidney contains large amounts of cathepsin B, which has been localized to renin-containing granules in the rat (13). However, the exact site of cleavage of prorenin by cathepsin B has not been identified. Thiol proteases, which cleave after dibasic residues, have been implicated in the processing of other mammalian prohormones such as proinsulin (38) and proalbumin (39). Thus, the RPE we have identified could be cathepsin B.

In summary, we have identified and partially purified a potential RPE in human kidney with the requisite properties for the authentic RPE. Ultimate confirmation of the enzyme we have characterized as the RPE lies in complete purification and identification of the enzyme, as well as localization of this enzyme to the renin-containing granules in the juxtaglomerular cells of the kidney.

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1. Sealey, J. E., Atlas, S. A. & Laragh, J. H. (1980) *Endocrinol. Rev.* **1**, 365–391.
2. Goldstone, R., Hsueh, W. A., Carlson, E. J. & Horton, R. (1983) *J. Clin. Endocrinol. Metab.* **56**, 264–268.
3. Page, I. H. & Bumpus, F. J. (1974) *Angiotensin: Handbook of Experimental Pharmacology* (Springer, New York), Vol. 37.
4. Luetscher, J. A., Kraemer, F. B., Wilson, D. M., Schwartz, H. C. & Bryer-Ash, M. (1985) *N. Engl. J. Med.* **312**, 1412–1418.
5. Hirose, S., Kim, S.-J., Miyazaki, H., Park, Y.-S. & Murakami, K. (1985) *J. Biol. Chem.* **260**, 16400–16405.
6. Do, Y. S., Shinagawa, T., Tam, H. & Hsueh, W. A. (1987) *J. Biol. Chem.* **262**, 1037–1043.
7. Taugner, R., Murakami, K. & Kim, S. J. (1986) *Histochemistry* **85**, 107–109.
8. Inagami, T., Okamoto, H., Ohtsuki, K., Shimamoto, K., Chao, J. & Margolius, H. S. (1982) *J. Clin. Endocrinol. Metab.* **55**, 619–623.
9. Carilli, C. T., Wallace, L. C., Smith, L. M., Wong, M. A. & Lewicki, A. (1988) *J. Chromatogr.* **444**, 203–208.
10. Heinrickson, R. L., Hui, J., Zurcher-Neely, H. & Poorman, R. A. (1989) *Am. J. Hypertens.* **2**, 367–380.
11. Xiong, W., Chao, L. & Chao, J. (1989) *Kidney Int.* **35**, 1324–1329.
12. Barrett, A. J. & Kirschke, H. (1981) *Methods Enzymol.* **80**, 535–580.
13. Taugner, C., Biihrle, C. P., Nobiling, R. & Kirschke, H. (1985) *Histochemistry* **83**, 103–108.
14. Slater, E. E., Gounaris, A. D. & Haber, E. (1979) *Clin. Sci.* **57**, 93s–96s.
15. Fritz, L., Arfsten, A., Carilli, C., Baxter, J. & Ponte, P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4114–4118.
16. Hsueh, W. A., Do, Y. S., Shinagawa, T., Tam, H., Ponte, P. A., Baxter, J. D., Shine, J. & Fritz, L. C. (1986) *Hypertension* **8**, Suppl. 2, 78–82.
17. Do, Y. S., Sherrod, A., Lobo, R. A., Paulson, R. J., Shinagawa, T., Chen, S., Kjos, S. & Hsueh, W. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1957–1961.
18. McIntyre, G. D., Leckie, B., Hallett, A. & Szelke, M. (1983) *Biochem. J.* **211**, 519–522.
19. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) *J. Biol. Chem.* **256**, 7990–7997.
20. Imai, T., Miyazaki, H., Hirose, S., Hori, H., Hayashi, T., Kageyama, R., Ohkubo, H., Nakanishi, S. & Murakami, K. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7405–7409.
21. Soubrier, F., Panthier, J. J., Corvol, P. & Rougeon, F. (1983) *Nucleic Acids Res.* **11**, 7181–7190.
22. Hardman, J. A., Hort, Y. J. & Catanzaro, D. F. (1984) *DNA* **3**, 457–468.
23. Hobart, P. M., Fogliano, M., O'Connor, B. A., Schaefer, I. M. & Chirgwin, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5026–5030.
24. Yokosawa, H., Holladay, L. A., Inagami, T., Haas, E. & Murakami, K. (1980) *J. Biol. Chem.* **255**, 3498–3502.
25. Slater, E. E. & Strout, H. V., Jr. (1981) *J. Biol. Chem.* **256**, 8164–8171.
26. Galen, F. X., Devaux, C., Guyenne, T., Menard, J. & Corvol, P. (1979) *J. Biol. Chem.* **254**, 4848–4855.
27. Evan, D. B., Weighous, T. F., Cornette, J. C., Tarpley, W. G. & Sharma, S. K. (1987) *Biotechnology* **5**, 705–708.
28. Ishizuka, Y., Saito, M., Hori, H., Poorman, R. A., Yoshida, S. & Murakami, K. (1988) *Biochem. Biophys. Res. Commun.* **152**, 849–856.
29. Derkx, F. H. M., Verhoeven, R. P. & Wenting, G. J. (1978) *Clin. Sci. Mol. Med.* **55**, 1295–1365.
30. Re, R. N. (1984) *Arch. Intern. Med.* **144**, 2037–2041.
31. Saint-Andre, J. P., Rohner, V., Pinet, F., Rousselet, M. C., Bigorgne, J. C. & Corvol, P. (1989) *Histochemistry* **91**, 291–297.
32. Orci, L., Ravazzola, M., Amherdt, M., Madsen, O., Perrelet, A., Vassalli, L. D. & Anderson, R. G. W. (1986) *J. Cell Biol.* **103**, 2273–2281.
33. Shaw, K., Do, Y. S., Kjos, S., Anderson, P. W., Shinagawa, T., Dubeau, L. & Hsueh, W. A. (1989) *J. Clin. Invest.* **83**, 2085–2092.
34. Shinagawa, T., Do, Y. S., Baxter, J., Shine, J. & Hsueh, W. A. (1987) *Clin. Res.* **35**, 449A (abstr.).
35. Szego, C. M., Seller, B. J. & Smith, R. E. (1976) *J. Biochem.* **69**, 463–474.
36. Takahashi, S., Murakami, K. & Miyake, Y. (1982) *J. Biochem.* **91**, 419–422.
37. Luetscher, J. A., Bialek, J. W. & Grislis, G. (1982) *Clin. Sci. Mol. Med.* **A4**, 2149–2159.
38. Docherty, K., Hutton, J. H. & Steiner, D. F. (1984) *J. Biol. Chem.* **259**, 6041–6044.
39. Bathurst, L. C., Brennan, S. O., Carrell, R. W., Cousens, L. S., Brake, A. J. & Barr, P. J. (1987) *Science* **235**, 348–350.