Purification and Characterization of Human Truncated Prorenin†

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ABSTRACT: Posttranslational processing of enzymatically inactive prorenin to an active form participates in the control of the activity of a key system involved in blood pressure regulation, growth, and other important functions. The issue is complicated because renin can be produced by a number of tissues throughout the body, in addition to the kidney, but the mechanism by which they process prorenin to renin is unknown and difficult to determine because of the small amounts of renin present. In the juxtaglomerular cell of the kidney, a 43 amino acid prosegment is cleaved from the amino terminus of prorenin to generate renin of molecular weight 44 000 [Do, Y. S., Shinagawa, T., Tam, H., Inagami, T., & Hsueh, W. A. (1987) *J. Biol. Chem. 262*, 1037-1043]. Using human uterine lining or a recombinant human prorenin system, we employed the same approach as that used in kidney, ammonium sulfate precipitation at pH 3.1 followed by pepstatin and H-77 affinity chromatography or gel filtration, to purify to homogeneity a 45 500-MW totally active renin. The specific activity of the active truncated prorenin was 850 Goldblatt units (GU)/mg of protein for chorion–decidua renin and 946 GU/mg of protein for recombinant renin, both similar to that reported for pure human renal renin. Both forms of renin cross-reacted with an antibody generated against 44 000-MW pure human renal renin and with an antibody generated against a peptide identical to the carboxy-terminal one-third of the prosegment. Amino-terminal sequence analysis of truncated recombinant prorenin indicated that 11 residues of the carboxy terminus of the prosegment were still present on the active renin. Enzyme activity of the truncated prorenins was similar to that of human renal renin. Truncated prorenin could also be demonstrated in human amniotic fluid. These studies suggest that alternate forms of active renin can exist with at least the carboxy-terminal one-third of the prosegment still attached. They further suggest that for human prorenin to be enzymatically inactive it must contain more than just the carboxy-terminal one-third of the prosegment. The significance of the present study lies in the fact that (1) a truncated form of prorenin was isolated from a human tissue, chorion–decidua, in which enough prorenin may be activated locally to generate substantial amounts of angiotensin II and (2) the correlation of structure with function provides a critical understanding of the importance of the prosegment to renin enzyme activity.

Renin (EC 3.4.23.15) initiates a cascade of events that represents a major body mechanism to regulate blood pressure, sodium and potassium balance, growth, and other cardiovascular functions. Interest has focused on the biosynthesis and posttranslational processing of this enzyme, since its activity governs the production of angiotensin II, which ultimately mediates these functions (Page & Bumpus, 1974; Peach et al., 1987; Frolich et al., 1989). Renin is synthesized from a precursor, preprorenin, which is the primary translational product of renin mRNA (Catanzaro et al., 1983; Pratt et al., 1983). The 23 amino acid preprosequence is cleaved to form prorenin. Similar to its relatives in the aspartyl protease family, the prorenin prosegment renders the enzyme inactive (Hsueh & Baxter, 1991). Active human renal renin of MW 44 000 is processed from prorenin by cleavage of the 43 amino acid prosegment after two dibasic residues (Do et al., 1987). However, several lines of evidence suggest that alternative means of activating prorenin may exist. First, wide variability has been reported for the MW of human circulating active renin, ranging from 40 000 to 60 000 (Sealey et al., 1980). Whereas this variability has been largely attributed to differences in glycosylation of the renin isozymes and techniques of measurement of MW, Hirose et al. (1985) presented immunological data suggesting that truncated forms of prorenin circulate in man. Second, it has been reported that the ischemic human kidney releases truncated prorenin (Pratt et al., 1987a,b). Furthermore, Hackenthal et al. (1990) found that immature secretory granules in human kidney juxtaglomerular cells demonstrated positive immunostaining with carboxy-terminal prosegment antibodies, but not with midportion prosegment antibodies, and concluded that prorenin may be converted to active renin by cleavage of only a portion of the prosegment. Third, there is a large body of evidence now implicating the activity of the tissue renin–angiotensin systems as important for regulating cardiovascular function. For example, studies with renin inhibitors indicate that blood pressure does not correlate with blockade of circulating renin activity (Anderson et al., 1990).

Prorenin mRNA have been detected in extrarenal sites such as ovary, adrenal, decidua, testes, pituitary, and heart (Deschepper et al., 1986; Do et al., 1988; Doi et al., 1984; Fernandez et al., 1985; Glorioso et al., 1986; Hirose et al., 1985; Lindpaintner et al., 1987; Pandey et al., 1984; Saint-Andre et al., 1989; Shaw et al., 1989;). Renin and prorenin from plasma can also be taken up by tissues such as heart, vascular wall, liver, and kidney (Louden, 1983; Skinner, 1986). Whereas some extrarenal tissues may process prorenin in a manner similar to that in the kidney, as is observed in cultured mouse AtT-20 cells (Fritz et al., 1987; Pratt et al., 1987a,b), other tissues such as placenta and ovary appear to release dominantly prorenin (Shaw et al., 1989; Do et al., 1988). That extrarenal tissues secrete primarily prorenin is further sug-

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gested by the finding that prorenin, but little active renin, can be found in the blood of anephric patients (Derk, 1978). Thus, the relative role of renin taken up from the circulation vs renin produced locally and how locally produced prorenin is activated remain important issues. More information is needed to determine whether prorenin can be processed by alternative cleavage mechanisms and whether such change would result in active renin. Since the mechanisms by which the prosegment of human renin inactivates the zymogen are not defined, information about truncated forms of prorenin would also be relevant to deciphering this phenomenon.

Chinese hamster ovarian (CHO) cells transfected with a human renin expression vector and cultured human chorion–decidua primarily release inactive prorenin (Fritz et al., 1986; Shaw et al., 1989). However, in the present investigation we have found that a large molecular weight form of active renin can be isolated from ammonium sulfate precipitates of the media of transfected CHO cells and of homogenates of chorion–decidua tissue. The recombinant active renin contained 11 amino acids of the prosegment as determined by amino-terminal sequencing. Chorion–decidua active renin also contained part of the carboxy terminus of the prosegment. This form of renin was also identified in amniotic fluid. Thus, enzymatically active forms of renin can exist which contain up to 11 residues of the prosegment. These results suggest that (1) posttranslational processing of prorenin to generate active renin may occur by several mechanisms and (2) enzyme activity, in addition to molecular weight, must be used to differentiate inactive prorenin from active forms of renin.

Experimental Procedures

Materials

Reagents were obtained from the following suppliers: ethylendediaminetetraacetate (EDTA), bovine serum albumin, phenylmethanesulfonyl fluoride (PMSF), mercaptoethanol, silver nitrate, Coomassie blue from Sigma (St. Louis, MO); 1,2-dimercaptopropane from Hynson, Mescott and Dunning (Baltimore, MD); 8-hydroxyquinoline (8OHQ) from Matheson, Coleman and Bell (Gibbstown, NJ); aprotinin from Pharmacia (Piscataway, NJ); H77 from Bachem (Torrance, CA).

Recombinant prorenin was obtained from culture media of Chinese hamster ovarian cells transfected with an expression vector for human preprorenin (Fritz, 1986). Human chorion–decidua tissue was dissected from the placenta and fetal membranes immediately after delivery and frozen at −20 °C until use. Human amniotic fluid was obtained from normal pregnant women following informed consent at the time of Caesarean section for delivery and frozen at −20 °C.

Methods

Renin Concentration. Renin concentration was determined by radioimmunoassay of angiotensin I (AI) generated by incubation with semipurified sheep angiotensinogen, final concentration 1.4 μM, at pH 7.4, 37 °C, for 10–30 min in the presence of 5 mM EDTA, 1.6 mM 1,2-dimercaptopropane, and 3.4 mM 8-hydroxyquinoline (Hsueh et al., 1983). In this assay, generation of 1.2×10 AI/mL h^{-1} equal to 1 Goldblatt unit (GU) of renin, was determined against Medical Research Council (MRC) renal renin (68–356) from the National Institute of Biological Standards and Controls, Holly Hill, London.

Protein Concentration. Protein was measured by the Folin-phenol method of Lowry et al. (1951) using bovine serum albumin as standard and by the Coomassie blue dye method of Bradford (1976). Absorbance at 280 nm was used to follow protein elution during chromatographic procedures.

Purification of Truncated Prorenin. Recombinant prorenin was purified using the following steps.

Acidification and ammonium sulfate precipitation: Culture media containing recombinant prorenin (Fritz, 1986) was concentrated approximately 40-fold using a Pellican concentrator (Millipore Model 4200K50). The concentrate was acidified to pH 3.1 with 5 N H_2SO_4 and made 0.8 M in NaCl after addition of protease inhibitors (10 mM EDTA, 1 mM PMSF, 0.4 mM 8OHQ, 20 000 kallikrein inhibitor units/L of aprotinin, and 20 mM benzamidine). All procedures were performed at 4 °C unless otherwise specified. After centrifugation (10 000 rpm for 30 min), the supernate was brought to 65% ammonium sulfate saturation and centrifuged, and the precipitate was dissolved in 20 mM histidine, pH 6.4, and dialyzed overnight against the same buffer.

Chromatofocusing: The dialyzed sample was applied to a 2.5×20 cm column of PBE-74 (Pharmacia) and eluted with poly buffer (Pharmacia) pH 4.0–6.2 at a flow rate of 70 mL/h. Twelve-milliliter fractions were collected. The renin-containing fractions were pooled (130 mL) and dialyzed against 10 mM sodium acetate, pH 6.5, containing 2 mM EDTA, 0.4 mM PMSF, and 0.4 mM benzamidine.

Pepstatin affinity chromatography: The dialyzed renin sample from the chromatofocusing column was applied to a 2.5×10 cm column of activated CH-pepstatin conjugated (to aminohexyl)-Sepharose as previously described (Do et al., 1987). The column was then washed with 150 mL of the above dialysis buffer containing 1 M NaCl and then 150 mL of 0.1 M Tris–acetate, pH 6.5, with 0.5 M NaCl. Renin was eluted with a linear pH gradient established with 200 mL of 0.15 M Tris–acetate containing 0.5 M NaCl, pH 6.5, in the mixing chamber and 200 mL of 0.15 M Tris–acetate containing 0.5 M NaCl, pH 8.5, in the reservoir applied with a flow rate of 40 mL/h. Six-milliliter fractions were collected. The renin-containing fractions were pooled (18 mL) and concentrated (Amicon, Danvers, MA).

Gel filtration: 100 μL of concentrated pooled fractions containing 1.3 mg of protein was applied to a Bio-Rad (Hercules, CA) high-pressure liquid chromatography system using a TSK G-3000 SW (Altech, Deerfield, IL) column (7.5×300 mm) and eluted with 50 mM $NaHPO_4$/0.5 M NaCl buffer, pH 6, containing 2% (v/v) dimethyl sulfoxide (DMSO) as previously described (Hsueh et al., 1986). The flow rate was 0.5 mL/min, and 1.0-mL fractions were collected. Protein elution was monitored by absorbance at 280 nm. The gel filtration column was calibrated with thyroglobulin (670 000), γ-globulin (158 000), ovalbumin (45 000), myoglobulin (17 000), and vitamin B_1 (1350) (Bio-Rad).

The procedure used for purification of renin from human chorion–decidua was identical to that previously reported for human kidney renin (Do et al., 1987). Briefly, chorion–decidua (8 g) was homogenized in 8 L of buffer in the presence of protease inhibitors. Following centrifugation of the homogenate, the supernate was acidified to pH 3.1 and subjected to ammonium sulfate precipitation. The 65% fraction was dissolved, dialyzed, and subjected to successive pepstatin and H77 affinity column chromatographies.

Characterization

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Samples were heated at 60 °C for 20 min with or without 0.1 M mercaptoethanol and 5–10 μL of buffer containing 0.5% SDS; 10% glycerol was applied to each lane of the gel. Slab gels consisted of 7.5–12.5% acrylamide, 2.6% cross-linking agent, and 0.1% SDS (minie-

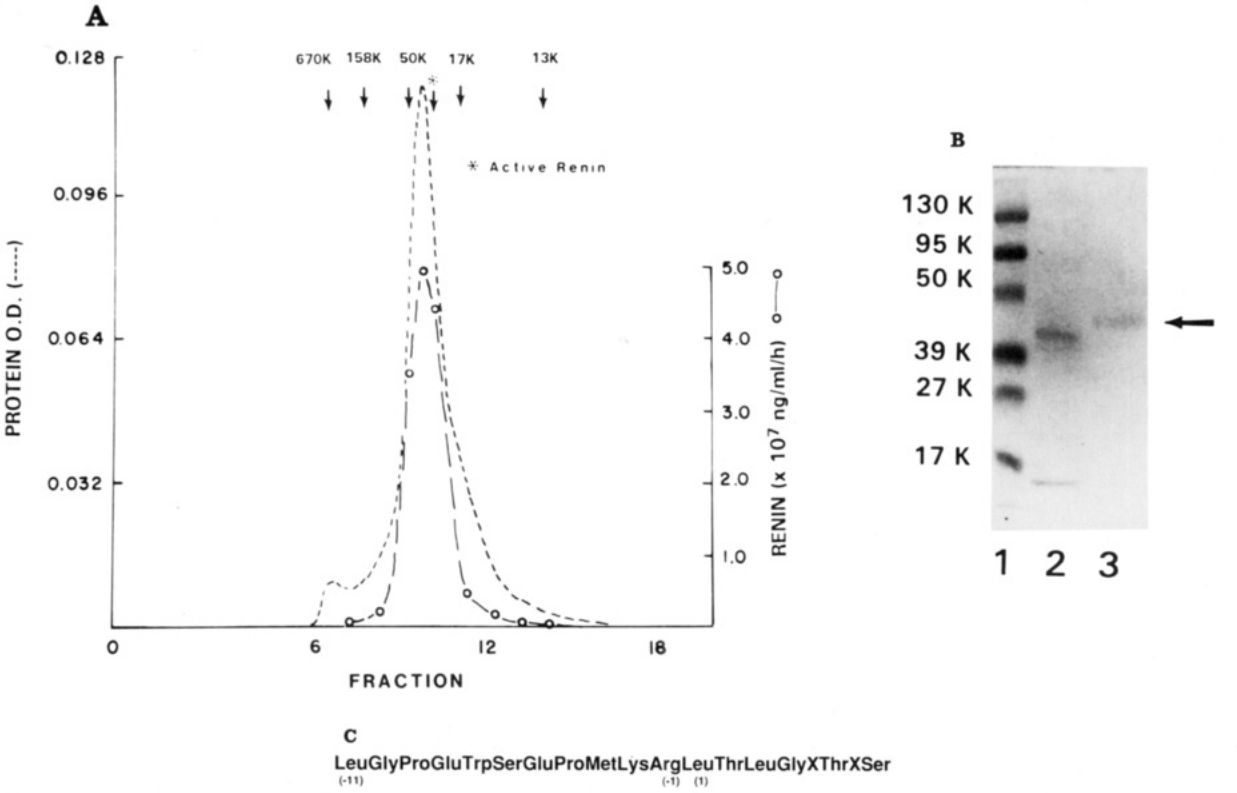

FIGURE 1: (A) Gel filtration elution of pure human truncated recombinant prorenin with a molecular weight of 45 500 which is larger than human renal active renin (44 000). Arrows denote molecular weight markers and active renin. (B) SDS–PAGE of pure human truncated recombinant prorenin (lane 3), human active renal renin (lane 2), and molecular weight markers (lane 1) stained with Coomassie blue. (C) Results of amino-terminal sequence analysis of pure human truncated recombinant prorenin; 19 cycles were obtained. Eleven amino acids of the prosegment are attached. X means undetermined and represents glycosylation sites at Asn.

lectrophoresis system, Bio-Rad). Electrophoresis was carried out at room temperature at 20–30 mA/slab gel for 4–5 h. Gels were stained with silver nitrate (Merril et al., 1983) or Coomassie blue (Bradford, 1976). The molecular weight was determined by comparison with protein standards (Bio-Rad) which included phosphorylase *b* (106 000), bovine serum albumin (80 000), ovalbumin (49 500), carbonic anhydrase (32 500), soyabean trypsin inhibitor (27 500), and lysozyme (18 500).

Kinetic Studies. Determination of pH optima and the Michaelis–Menten constant, K_m, were performed as previously described (Do et al., 1987) with both sheep and human angiotensinogen. Pure human angiotensinogen was kindly provided by Dr. Duane Tewksbury, Marshfield, Wisconsin (1976). Protease inhibitors were not added to the reaction between pure human renin and pure human renin substrate.

Immunoblot Analysis. This was performed after proteins were transferred from SDS slab gels into Whatman 3M nitrocellulose using the minitransblot system (Bio-Rad). The membrane was blocked by 5% BSA in TBS (10 mM Tris buffer containing 0.15 M NaCl, pH 7.4) and incubated with rabbit antibody either against human active renin (Do et al., 1987) or against the prosegment of renin, from Arg^{-1} to Lys^{-1} (Hsueh et al., 1986) at 1/200 dilution overnight at 4 °C. ^{125}I-Labeled protein A was used to detect proteins cross-reacting with antibodies (New England Nuclear, Boston, MA).

Amino Acid Sequencing. Following elution from H77, samples were concentrated, lyophilized, dissolved in water, and applied to an Applied Biosystems Model 370A gas-phase microsequencer (USC Cancer Center Microchemical Core Resource) according to Hewick et al. (1981). The PTH derivatives were analyzed by the on-line PTH analyzer (Applied Biosystems, Model 120).

Cibacron-Blue Binding. Intact human prorenin is known to bind to Cibacron-blue (Hsueh et al., 1986). The binding of truncated human prorenin was assessed as previously described in detail under the same conditions used for intact prorenin (Hsueh et al., 1986).

Results

Purification of Truncated Prorenin

CHO Media. Results of purification of truncated prorenin from CHO media are shown in Table I. In the starting material, 6.4% of the total renin was active. Ammonium sulfate precipitation at pH 3.3 resulted in almost total activation of the recombinant prorenin. During chromatofocusing, renin eluted in two peaks between pH 5.2 and 5.4 and between pH 4.6 and 5.0. Fractions eluting at the lower pH range, containing 80% of the active renin, were pooled and applied to a pepstatin affinity column. Renin eluted from the pepstatin column in a single symmetrical peak with a 0.1 M Tris-HCl, pH 8.5, wash, resulting in a 56-fold purification. No inactive prorenin was detected in the pass-through fractions. Gel filtration HPLC of this peak fraction, resulted in complete purification of an active form of prorenin with a specific activity of 946 GU/mg of protein (Figure 1A). The complete purification scheme yielded 0.65 mg of renin, which represented an overall 68-fold purification and a yield of 44%. Silver staining of pure active prorenin, run on SDS–PAGE, demonstrated a single band of protein with a MW of 45 500 which is larger than pure human active renin (Figure 1B) but smaller than intact prorenin (47 000). The latter has a MW of 44 000 with 22 000- and 18 000-MW subunits (Do et al., 1987).

When truncated recombinant prorenin was subjected to amino-terminal sequencing, 19 cycles were obtained. Comparison with the amino-terminal sequence of pure human renal

Table I: Results of Purification of Human Truncated Prorenin

	protein (mg)	active renin (GU)	total renin (GU)	active/total (%)	sp act. (GU/mg)	purification (x-fold)	yield (%)
			Human Truncated Prorenin from Chorion–Decidua				
chorion–decidua homogenate	114800	19.0	95.0	20.0	8.3×10^{-4}		
ammonium sulfate precipitation	4,500	57.8	63.7	90.7	0.014	16.9	67.1
pepstatin chromatography	4.9	42.0	52.6	79.8	10.7	12890	59.0
H77 chromatography	0.023	14.4	19.3	74.6	850	1.0×10^6	36.7
			Human Truncated Recombinant Prorenin				
CHO media	100.6	89.5	1399	6.4	13.9		
ammonium sulfate precipitation	34	1333	1392	95.8	40.9	2.9	99.5
chromatofocusing	2.04	1155	851	145.0	417.5	30.0	82.3
pepstatin chromatography	1/32	1090	1028	106.2	780.4	56.1	77.9
gel filtration	0.65	691	616	112.0	946.0	68.1	49.0

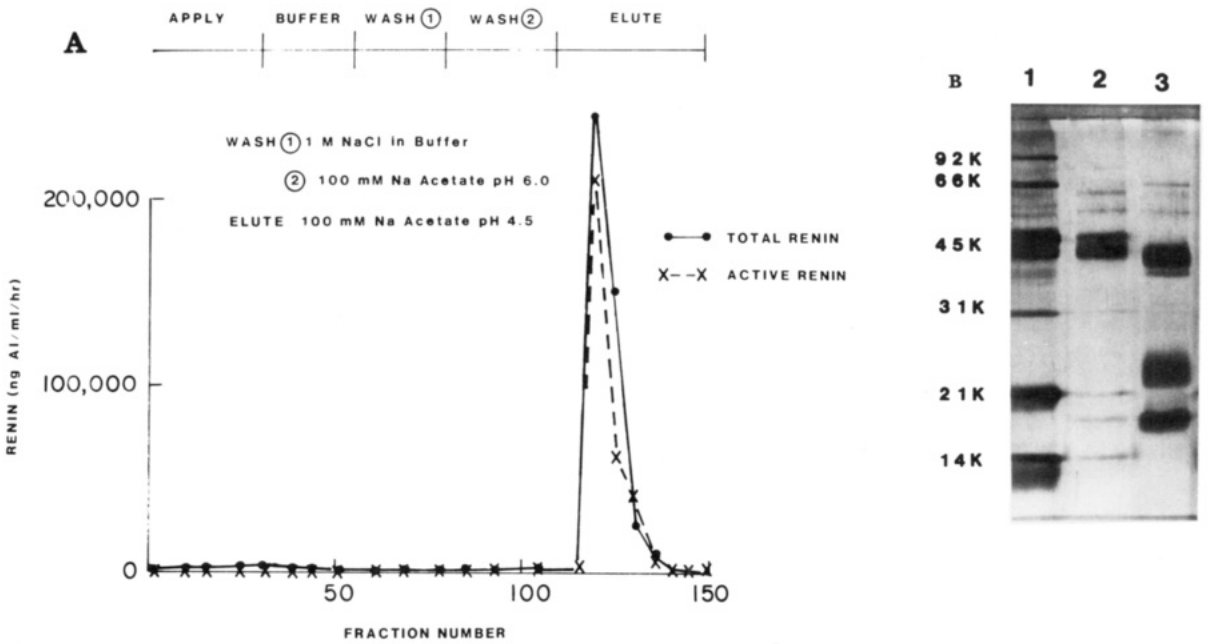

FIGURE 2: (A) Elution of human chorion–decidua truncated prorenin from an H77 affinity column. Prorenin in the eluates was completely active. (B) Silver nitrate stained SDS–PAGE of pure human chorionic–decidua truncated prorenin (lane 2) compared to active renal renin (lane 3). Molecular weight markers are in lane 1.

renin (Do et al., 1987) and the deduced amino acid sequence from the cDNA sequence of prorenin (Miyazaki, 1984) indicated that truncated prorenin had 11 amino acids of the prosegment attached to active renin beginning at Leu^{-11} (Figure 1C). The glycosylation residues Asn^5–Asn^7 were not detected, as previously reported for pure active renal renin (Do et al., 1987).

Human Chorion–Decidua. Eight kilograms of human chorion–decidua was homogenized in 8 L of buffer; 20% of the total renin in the homogenate was active. Subsequent ammonium sulfate precipitation at pH 3.3 resulted in complete activation of human inactive renin (Table I). All of the activated renin bound to pepstatin (aminohexyl)-Sepharose and was eluted in a single symmetrical peak with a 0.15 M Tris–acetate, pH 8.5, wash. H77 affinity chromatography of the pepstatin-bound fractions resulted in complete purification of activated human prorenin with a specific activity of 850 GU/mg of protein (Figure 2A). A total of 23 μg of the final product was obtained, which represented a 10^6-fold purification and 37% yield. Silver staining of the activated prorenin run on SDS–PAGE demonstrated a single band of protein of molecular weight 45 500 (Figure 2B).

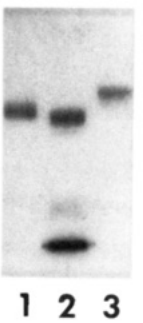

FIGURE 3: Immunoblot of human truncated prorenin (lane 1), pure human renal renin (lane 2), and intact human recombinant prorenin (lane 3) developed with an antibody against human renal renin. The molecular weights are 45 500, 44 000, and 47 000. Renal renin also exists as subunits of 22 000 and 18 000 (Do et al., 1987).

Characterization

Immunoblot Analysis. Truncated prorenin isolated both from CHO media and from the human chorion cross-reacted with the antihuman renal renin antibody indicating antigenic similarity to human kidney renin. The relative running rates

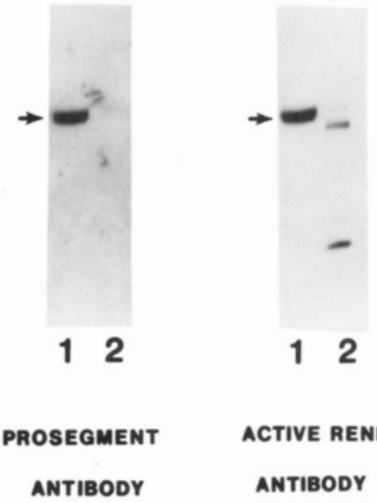

FIGURE 4: Immunoblot of chorionic and kidney renin. Human truncated prorenin (lane 1) cross-reacts with both the prosegment antibody and the renal renin antibody; renal renin (lane 2) only cross-reacts with the renal renin antibody. See text for details.

of recombinant prorenin, truncated recombinant prorenin, and human renal renin on a 7% SDS gel are shown on an immunoblot in Figure 3 developed with the antihuman renal renin antibody. The relative molecular weights are 47 000, 45 500, and 44 000, respectively. In Figure 4, truncated human chorion–decidua renin is shown to cross-react with the antihuman renal renin antibody as well as an antibody generated against the carboxy-terminal one-third of the prosegment of prorenin (Hsueh et al., 1986), while pure human renal renin does not cross-react with the prosegment antibody. These data suggest the molecular weights of truncated recombinant prorenin and truncated chorion–decidua prorenin are the same.

Enzyme Activity. Both forms of truncated prorenin were fully active when tested against pure human angiotensinogen or semipurified sheep angiotensinogen. The pH optimum of truncated recombinant prorenin were 6.0 and 7.5, and the K_m values were 1.3 and 0.2 μM with human and sheep substrate, respectively. The pH optimum of truncated chorionic prorenin were 6.0 and 7.5, and the K_m values were 1.4 and 0.16 μM, respectively, with human and sheep substrate. Thus, both forms of truncated prorenin had similar enzyme activity with two different substrates. Treatment of either the truncated prorenins with trypsin (10 μg/mL or 10 ng/mL) did not increase enzyme activity. Incubation of either preparation of pure truncated prorenin at 37 °C, pH 7.5, for 2 h did not result in loss of enzyme activity.

Cibacron-Blue Binding. Cibacron-blue binding affinity chromatography has been used as a standard technique to separate active from inactive forms of renin (Hsueh et al., 1983). Human inactive prorenin binds to Cibacron-blue while active renin does not bind. When applied under conditions known to bind inactive prorenin (Hsueh et al., 1986), neither form of truncated prorenin bound to a Cibacron-blue affinity column (data not shown).

Amniotic Fluid. Active renin constituted 15% of the total renin in amniotic fluid (535 ng/mL h^{-1} active/3576 ng/mL h^{-1} total). Ammonium sulfate precipitation at pH 3.1 resulted in 32% of the total renin being active (1144 ng/mL h^{-1}). Immunoblot analysis of human amniotic fluid is shown in Figure 5. The primary form of renin in amniotic fluid is 47 000-MW prorenin. After ammonium sulfate precipitation at pH 3.1, approximately 20% of the intact prorenin appears to be converted to truncated prorenin. Little 44 000-MW renin is detectable.

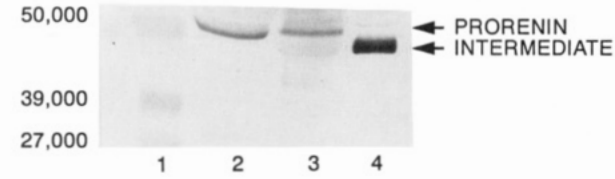

FIGURE 5: Immunoblot analysis of human amniotic fluid before (lane 2) and after (lane 5) ammonium sulfate precipitation at pH 3.1. Molecular weight standards are in lane 2. There is generation of detectable truncated prorenin after treatment (see text).

DISCUSSION

In this investigation we have isolated and purified to homogeneity a fully active truncated form of human prorenin. The sources of truncated prorenin included both a cell culture system, developed to produce human recombinant prorenin, and a native human tissue, known to produce prorenin. The major renin product of both cultured CHO cells transfected with the human renin gene and cultured human chorion–decidua cells is 47 000-MW inactive prorenin (Fritz, 1986; Hsueh et al., 1986; Shaw et al., 1989). Human recombinant or native inactive prorenin can be activated either by limited proteolysis or by exposure to low pH (Sealey et al., 1980; Hsueh et al., 1981). In our procedure, prorenin was activated and likely cleaved by an enzyme(s) in the CHO cell media or chorion–decidua homogenate during ammonium sulfate precipitation at low pH. Intact, inactive recombinant prorenin can be purified from CHO media if exposure to low pH (i.e., <3.5) is avoided (Shinagawa et al., 1990). Intact, prorenin has also been purified from human chorion–decidua (Higashimori et al., 1989). In our studies, truncated recombinant prorenin was cleaved between Arg^{-12} and Leu^{-11}. Chorion–decidua renin that we isolated had a molecular weight identical to that of truncated recombinant prorenin. Both forms of renin cross-reacted with antibodies generated against the prosegment of prorenin, confirming that renin isolated from human decidua also contained a portion of the prosegment and, hence, is a truncated form of native prorenin. The enzyme responsible for partial cleavage of the prosegment in our system is unknown. Egan et al. (1988) have identified cathepsin B in their recombinant system which activates prorenin at low pH. However, we demonstrated that cathepsin B correctly cleaves the prosegment of prorenin and that it appears to be the prorenin-processing enzyme in human kidney (Wang et al., 1991).

In the present study, we employed a procedure to purify renin from human chorion–decidua tissue identical to that we employed for purification of renin from human kidney, which also involved ammonium sulfate precipitation at low pH (Do et al., 1987). Despite use of an identical technique, we isolated an active renin from pregnancy tissue which is larger in molecular weight than active kidney renin and which contains part of the prosegment. Thus, chorion–decidua has an enzyme which activates prorenin but processes it differently from the major pathway used by the kidney. Cathepsin B in human kidney homogenate activates recombinant prorenin at pH 6.0, 37 °C, and cleaves the entire prosegment (Shinagawa et al., 1990; Wang et al., 1991). Under these conditions, chorion–decidua homogenate did not process recombinant prorenin (Shinagawa et al., 1990). Thus, extrarenal tissues which synthesize prorenin have different processing capabilities and utilize different mechanisms to activate prorenin than the kidney. Despite the size difference, the enzyme activity of pure active truncated human prorenin and human kidney appeared similar. The pH optima and K_m of truncated renin from both sources, with either sheep or human substrate, are generally

similar to the values obtained for the reaction of human renal renin with both substrates (Do et al., 1987). In addition, both truncated forms of renin were inhibited by H77, a specific inhibitor of active renal renin. Thus, although extrarenal and renal processing of prorenin may be different and may involve enzymes that cleave different portions of the prosegment, the active renin that results has a similar ability to generate angiotensin I.

The current finding of an alternative prorenin cleavage activity in human chorion–decidua indicates that this tissue has the potential to generate active renin. Levels of renin gene expression in chorion decidua approach those seen in kidney (Shaw et al., 1989), suggesting chorion–decidua is a major source of human prorenin. In fact, prorenin from chorion–decidua likely collects in amniotic fluid, which contains 10–20-fold the prorenin concentrations seen in the circulation (Lumbers, 1971). We also identified the presence of truncated prorenin in human amniotic fluid, suggesting that truncated prorenin may be generated in both decidual tissue and amniotic fluid in pregnant women. Because of the large quantities of prorenin in uterine lining and amniotic fluid, some activation of prorenin in this system could have extensive physiological impact. If large quantities of truncated prorenin were readily detectable in amniotic fluid or plasma by immunoblot analysis, a tremendous amount of active renin would be present. Thus, our data do not allow us to know whether this mechanism of activation occurs in vivo because plasma and amniotic fluid do not contain high enough levels of active renin to directly assess size by Western blotting. Previous studies also point to other potential mechanisms for activating prorenin. Trypsin can cleave recombinant prorenin to generate several truncated enzymatically active prorenins with as much as 29 amino acids of the prosegment attached (Heinrikson, 1989). Thus, a seemingly wide span of prorenin cleavage sites within the prosegment is potentially available for tissue generation of active renin and, hence, local activation of the renin–angiotensin cascade.

Our observation that as many as 11 amino acids of the prosegment attached to renin still allow the enzyme to maintain full activity is consistent with previous studies demonstrating that inhibitory activity of the prosegment of prorenin lies in the amino-terminal two-thirds of the prosegment (Evan et al., 1984; Cumin et al., 1985). Like other aspartyl proteases, i.e., pepsinogen and penicillopepsin, computer-generated three-dimensional structure analysis suggests that the prosegment of prorenin covers the active site to maintain the enzyme in an inactive state (Baxter et al., 1991). In the mouse 45 amino acid prosegment of submaxillary gland renin, inhibitory activity was localized to amino acids 15–19 of the prosegment (Evan et al., 1984). In human prorenin, inhibitory activity was localized to amino acids 16–20 of the prosegment (Cumin et al., 1985). Localization was determined by synthesis of peptides corresponding to different areas of the prosegment and determining their renin inhibitory potency. Acid activation is reversible, unless intact prorenin is cleaved during the acid exposure (Hsueh et al., 1981). Enzyme activity of truncated prorenin is not reversible, further suggesting that the amino-terminal two-thirds of the prosegment must be present to allow the conformational change that occurs with acid activation and its reversibility. Inactive prorenin binds to Cibacron-blue, but truncated active prorenin and renal renin do not bind. Thus, the Cibacron-blue binding site must also reside in the amino-terminal two-thirds of the prosegment. X-ray crystallography of human prorenin will be important in determining how the prosegment interacts with the active site of renin and in more precisely defining the inhibitory areas of the prosegment (Hsueh & Baxter, 1991).

The present studies further emphasize the importance of clearly defining "prorenin". Intact prorenin is the biosynthetic precursor of renin and has little intrinsic capability of angiotensin I generation. Functional forms of prorenin can be generated in vitro, which can possess full enzyme activity. Indirect immunological evidence suggests these forms may exist in vivo, but definitive evidence is lacking. Using a battery of antibodies directed against different thirds of the prosegment, Hirose et al. (1985) concluded that human circulating prorenin is truncated because only the antibody directed against the carboxy-terminal one-third of the prosegment bound plasma prorenin. Yet, our studies demonstrate that this truncated form of renin is fully active. It is possible that antibodies directed against the first two-thirds of the prosegment in these studies did not bind to inactive prorenin because this portion of the prosegment may be buried in the active site. Nevertheless, the presence of portions of the prosegment on the renin molecule does not necessarily indicate that the renin is inactive. Thus, ability to generate angiotensin I or the use of active site directed antibodies will be necessary to assess enzyme activity.

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