Biochimica et Biophysica Acta 965 (1988) 68–75
Elsevier

BBA 22903

Human placental chorionic renin: production, purification and characterization

David A. Egan, Valerie Grzegorczyk, Karen A. Tricarico, Annemarie Rueter, William H. Holleman and Patrick A. Marcotte

Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL (U.S.A.)

(Received 15 November 1987)

Key words: Renin; Aspartyl proteinase; Angiotensin

Native human renin, produced from the culture of human chorionic trophoblasts, has been purified to homogeneity on a milligram scale using a five-step purification scheme. The chorion cells secrete 50–200 milliGoldblatt Units of trypsin-activatable prorenin per ml into the medium. The pro-enzyme is partially purified by ammonium sulfate fractionation and chromatographies on QAE-Sephadex and cibracon blue-agarose. Following conversion of prorenin to the active enzyme by porcine trypsin, the renin is purified to homogeneity by affinity chromatography and gel filtration. Chorionic prorenin has a molecular weight of 43000; the active enzyme 40000. Both proteins exist as a single polypeptide chain as determined by SDS-polyacrylamide gel electrophoresis under reducing conditions. The average specific activity of six different preparations was found to be 1072 Goldblatt Units/mg. The amino acid composition and N-terminal sequence of the active enzyme has been determined and is identical to the human kidney enzyme. Microheterogeneity of chorionic renin was demonstrated by isoelectrofocusing analysis. The physical characterization of chorionic renin is compared with that reported for the human kidney enzyme.

Introduction

Renin is an aspartyl proteinase which cleaves the N-terminal decapeptide from the α-2 globulin, angiotensinogen. The decapeptide, angiotensin I, is further processed by angiotensin-converting enzyme, a metallocarboxydipeptidase, to the octapeptide angiotensin II, a potent vasoconstrictor and a regulator of sodium [1]. Inhibitors of angiotensin-converting enzyme have been found to be efficacious for antihypertensive therapy [2], and therefore the inhibition of renin is a subject of wide research interest [3–6].

Renin has been found in many tissues and fluids including kidneys [7–11], blood plasma [12], brain [13], amniotic fluid [14], amnion [15], and placental chorion [14,16]. The partial or total purification of renin has been reported using kidneys [7–11], blood plasma [12], juxtaglomerular cell carcinomas [17] and placental chorion [18]. A recent report used Chinese Hamster Ovary cells transfected with human preprorenin cDNA as a source of renin [19].

We have devised a method using chorionic cell culture as the source of prorenin. Chorionic trophoblasts are known to secrete prorenin into the medium when grown in culture [20]. Under the conditions outlined in this report, these cells secrete 50–200 milliGoldblatt Units (mGU) per ml of trypsin-activatable prorenin into the culture

Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Mops, 4-morpholinepropanesulfonic acid; Boc, butoxycarbonyl.

Correspondence: P.A. Marcotte, Abbott Laboratories, Cardiovascular Biochemistry, D-47B, AP10, Abbott Park, IL 60064, U.S.A.

medium. (One mGU is defined as that amount of enzyme which produces 120 ng of angiotensin I per ml per h at 37°C by RIA vs. the Medical Research Council angiotensin I standard.) These cells continue to secrete these levels of prorenin for several months. We have found that the culture medium containing the prorenin can be stored frozen and is stable for at least a year. After ammonium sulfate fractionation, there are two chromatographic steps on the prorenin. Trypsin activation, which converts the prorenin to active enzyme, followed by two subsequent chromatographic steps gives purified renin on a milligram scale. The renin has a molecular weight of 40 000 and is homogeneous by SDS-PAGE. The average specific activity of six different preparations of purified renin was determined to be 1072 Goldblatt Units/mg. The properties of activated human chorionic renin are presented in this paper and are compared to those reported for the enzyme derived from human kidneys.

Experimental procedures

Materials

Collagenase class IV was from Cooper Biomedical, DNAase from Calbiochem, Hanks' Solution and CMRL 1066 Medium from Gibco Laboratories, NuSerum from Collaborative Research, and gentamycin from MA Bioproducts. Trypsin, soybean trypsin inhibitor, and neuraminidase were from Sigma, endoglycosidases F and H from Dupont, and endoglycosidase D from Seikaku Biochemicals. QAE-Sephadex and AH-Sepharose were purchased from Pharmacia, cibracon blue-agarose (Affi-gel blue) and Western blot reagents from Bio-Rad Laboratories, and Ultrogel AcA-44 from LKB. Ampholytes and isoelectric focusing (IEF) standards were purchased from Pharmacia. Calibrated human kidney renin standard was purchased from USBC.

Methods

SDS-PAGE in slabs were performed using a modified Laemlli protocol [21]. Acrylamide gel electrofocusing in tubes was done as described by Righetti and Drysdale [22]. Protein determination was by the method of Lowry et al. [23] with bovine serum albumin used as the standard. Angiotensinogen was purified from human plasma by a modification of the method of Dorer et al. [24] and Tewksbury et al. [25]. Angiotensinogen used in kinetic analyses was homogeneous by SDS-PAGE and released 18 μg angiotensin I per mg of protein upon exhaustive treatment with renin. Amino acid composition of renin was determined using a Waters Workstation subsequent to hydrolysis for 2 h in 5.7 N HCl, 0.03% phenol, 0.1% β-mercaptoethanol at 150°C under vacuum. Automated Edman degradation was performed using an Applied-Biosystem Model 470A vapor phase protein sequencer.

Culture method

Human placentas obtained within 30 min of delivery were transported in sterile chorion buffer (Hanks' Balanced Salt Solution, 10 mM Mops, 10 mM dextrose, 25 μg/ml gentamycin, pH 7.0). The amnio-chorio layer was removed from the maternal side of the placenta, and washed repeatedly with the chorion buffer. The amnion was stripped off and discarded. The chorion was washed, residual clots eliminated, and the chorion refrigerated overnight in chorion buffer. The following day the chorion was minced and treated with collagenase class IV (1 mg/ml) and DNAase (30 μg/ml) in chorion buffer for 3 h at 37°C. Cells were harvested, red blood cells were lysed, and the cells were counted for viability. The cells were planted in 150 ml tissue culture flasks at 30 million cells/flask in CMRL 1066 medium supplemented with 15% NuSerum and 25 μg/ml gentamycin. At confluency, the NuSerum content was reduced to 6%. The medium was changed twice weekly, with samples taken for renin assay. The decanted medium containing varying amounts of prorenin was stored at −70°C. Chorion media solutions found to contain less than 50 mGU/ml of prorenin were discarded.

Trypsin activation assay

Samples were assayed after dilution in 5% bovine serum albumin. Complete activation was achieved by incubation of 20 μl of diluted sample with 50 μl of a 10 μg/ml porcine trypsin solution in 50 mM Mops, 200 mM NaCl, 1 mM EDTA, pH 6.5 for 30 min at room temperature. The activation was terminated by addition of 10 μl of

2 mg/ml soybean trypsin inhibitor in buffer. The renin assay was initiated by the addition of 20 μl of a solution of partially purified human angiotensinogen. After a 2 h incubation at 37 °C, 10 μl was removed, diluted into 40 μl 5% bovine serum albumin, and the angiotensin I produced was determined with a modification of the method of Haber et al. [26] using the Dupont/New England Nuclear assay kit. Angiotensin I generation was converted to mGU of renin activity by comparison with a commercially available calibrated renin (assay range 1.56–25 mGU/ml). Chorionic culture supernatants, column fractions, and partially purified preparations were assayed for their prorenin activity in this manner. The assay was adapted to the requirements of CETUS automatic pipetter to facilitate sample analysis.

Activation of prorenin was also attempted using urinary or plasma kallikrein of human origin. Using conditions as outlined for the trypsin activation step, we were not able to convert the prorenin to active enzyme.

Enzyme purification

All steps, except for trypsin activation (step 4) were carried out at 0–4 °C. Buffers were adjusted to the indicated pH at room temperature.

Step 1. Frozen chorionic culture supernatants containing 50–200 mGU prorenin activity per ml were thawed and fractionated with ammonium sulfate (40–75% saturation). The pellet was reconstituted with a minimal volume of 20 mM Tris-HCl, 140 mM NaCl, pH 7.9 (buffer A) and dialyzed vs. three changes of the same buffer.

Step 2. The dialyzed protein solution (300–400 ml) was passed through a 5 × 50 cm column of QAE-Sephadex equilibrated with buffer A. The column was developed isocratically with buffer A until the prorenin activity was eluted (approximately three column volumes).

Step 3. The eluate from the QAE-Sephadex column (2500 ml) was diluted to 100 mM NaCl with 50 mM Tris-HCl, 1 mM EDTA, pH 7.5 (buffer B) and eluted onto a 2.5 × 40 cm column of cibracon blue-agarose (Affi-gel blue). After washing with buffer B containing 100 mM NaCl, the prorenin was eluted with buffer B containing 1 M NaCl. The fractions containing prorenin were pooled and concentrated to 5–10 mg/ml protein by ultrafiltration with an Amicon PM-10 membrane. The solution was dialyzed vs. 30 mM sodium phosphate, 100 mM NaCl, pH 6.9 (buffer C). The preparation can be stored at −70 °C at this stage.

Step 4. Porcine pancreatic trypsin was added to the prorenin solution at a final trypsin concentration of 10 μg/ml. After 30 min at room temperature, the solution was placed in an ice bath and a 20-fold weight excess of soybean trypsin inhibitor was added. The solution was then eluted onto a 1 × 10 cm AH-Sepharose column to which the renin inhibitor Boc-Phe-His-Leu^{R}-Val-Ile-His had been attached [27]. The column was successively washed with buffer C, buffer C with 1 M NaCl, and 0.1 M sodium acetate, until the ultraviolet absorbance at 280 nm was zero. The renin was eluted with a 200 ml gradient of 0.1 M sodium acetate to 0.1 M acetic acid and it eluted as a symmetrical peak at pH 5.0. Active fractions were pooled, neutralized to pH 6.5 with sodium phosphate, concentrated by vacuum dialysis, and dialyzed vs. buffer C.

Step 5. The protein from step 4 was passed through a 4 × 100 cm column of Ultrogel AcA-44 equilibrated and eluted with buffer C. Active fractions were pooled and concentrated to approximately 1 mg/ml protein by vacuum dialysis. The preparation was frozen in liquid nitrogen and stored at −70 °C.

Endoglycosidase experiments

Purified human renin (1.1 mg/ml) in 30 mM sodium phosphate, 100 mM sodium chloride, pH 6.9 was incubated with neuraminidase (3.2 units/ml), endoglycosidase D (0.12 units/ml), endoglycosidase F (4 units/ml), or endoglycosidase H (8.9 μg/ml). The amounts of endoglycosidase chosen for these experiments were chosen in relation to the activity of the commercial preparations. After 2 h of incubation at 37 °C, 25 μl of the incubation mixture was added to 25 μl of saturated sucrose and subjected to acrylamide gel electrofocusing in 4 mm i.d. tubes using ampholytes of pH 4–6.5.

Antibody preparation and characterization

Affinity-purified human chorionic renin was homogenized in complete Freund's adjuvant and

injected intradermally (100 μg/animal) into rabbits. The animals were reinjected with renin in incomplete adjuvant at monthly intervals. The antiserum used in the Western blot analyses (Fig. 3) was from an animal who had received three monthly injections. Although affinity-purified renin was used as antigen, significant cross-reactivity of the antiserum to other components in the culture medium was observed (Fig. 3, lane B). This cross-reactivity did not interfere with the use of the antiserum in the Western blot studies of the conversion of prorenin to renin (Fig. 3, lanes C–F).

Results

Cell culture

We have found that primary placental chorionic cells can be cultured and will secrete prorenin into the medium. The enzyme activity of a culture plotted over time (Fig. 1) shows that over 90% of the enzyme is in the pro-form. Prorenin production is evident in the first 10 days of culture, peaks in the first 3 weeks, and slowly declines over several months. Preparations usually continue to secrete high levels of prorenin for several months. Culture fluids containing over 50 mGU/ml trypsin-activatable renin activity are pooled and stored frozen at −70 °C until purification is initiated. An immunoreactive band of M_r 43 000 is visible in active culture supernatants. We have found that milligram quantities of human prorenin can be produced from the culture of a single chorion.

Purification

Our protocol, which involves five steps and an overall yield of 60%, is summarized in Table I. Ammonium sulfate fractionation affords a 3-fold purification and a 50-fold reduction in volume. Filtration through QAE-Sephadex achieves a 4-fold enhancement of specific activity. The protocol then utilizes the affinity of the pro-enzyme for cibracon blue-agarose. Human prorenin from amniotic fluid, chorionic membranes, plasma, and kidney has been shown to be adsorbed to this

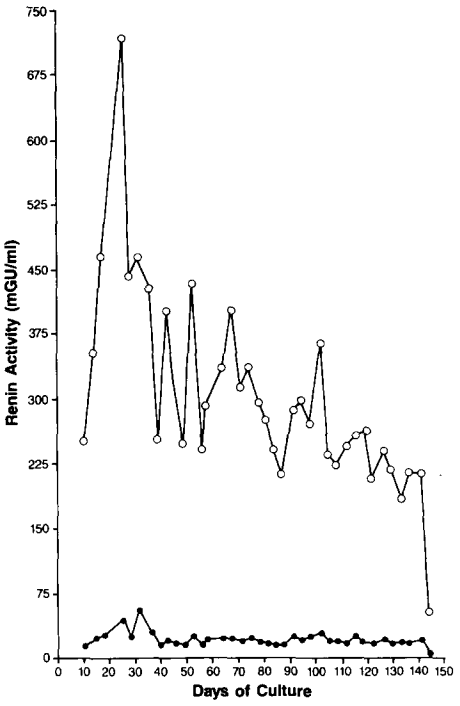

Fig. 1. Renin activity secreted by human chorionic trophoblasts over time. ●, active renin; ○, total renin. For assay conditions see text.

TABLE I

SUMMARY OF THE PURIFICATION OF HUMAN CHORIONIC RENIN

Protein was determined by the method of Lowry et al. [23], with bovine serum albumin as a standard. n.d., not determined.

	Volume (ml)	Protein (mg)	GU/ml	GU/mg	Total GU	Purification (fold)	Yield (%)
Start	23550	37680	0.48	0.30	11210	1	100
Ammonium sulfate	383	12245	27.96	0.90	10708	3	96
QAE-Sephadex	2240	2262	3.61	3.58	8081	12	72
Affi-gel blue	63	312	125.81	25.43	7927	85	71
Affinity *	3	n.d.	n.d.	n.d.			
AcA-44	10	6	681.78	1072	6818	3573	61

* BOC-Phe-His-Leu^R-Val-Ile-His–AH-Sepharose.

support at low salt concentrations and eluted with buffers containing molar concentrations of sodium chloride [14,28,29]. The QAE-Sephadex/cibracon blue column sequence is a modification of the method of Atlas et al. [12] used for the partial purification of prorenin from human plasma.

After concentration of the eluate from the cibracon blue column to 5–10 mg/ml protein, and dialysis vs. low-salt buffer, the enzyme is activated by the addition of porcine pancreatic trypsin. The optimal concentration of trypsin is usually 10 μg/ml at room temperature or 4 μg/ml at 37 °C. At the optimal concentration, little loss of activity is observed on prolonged incubation (Fig. 2), indicative of a stable proteolyzed product. We have found that a higher concentration of trypsin results in rapid activation but substantial degradation upon longer incubation times. The activation can also be followed (Fig. 3, lanes C–F) by Western blot analysis of immunoreactive renin. The 43 000-M_r species (prorenin), following trypsin activation, is converted to 40 000-M_r (active renin). A small amount of over-proteolyzed enzyme is evident at M_r 28 000 (Fig. 3, lanes E and F). After activation, the solution is chilled and an excess of soybean trypsin inhibitor is added. The solution is then passed through an AH-Sepharose column to which the inhibitor Boc-Phe-His-Leu^R-Val-Ile-His has been attached. The chorionic enzyme adsorbs and elutes from the affinity support in the same manner as described by Stein et al. [27] for use of this support in the purification of the human kidney enzyme. After extensive washing, the active enzyme is eluted with an acetic acid gradient (pH of elution, 5.0). The final gel filtration step (Ultrogel AcA-44) results in a homogeneous preparation as determined by SDS-PAGE under reducing conditions.

Characterization

The renin is a single band on SDS-PAGE (Fig. 4) under both reducing and non-reducing conditions, with a molecular weight of 40 000. Our preparations do not contain high amounts of the lower molecular weight species that have been reported in preparations of the kidney enzyme [8,11], although the smaller immunoreactive renin band appears to have some capacity to bind to the affinity column (Fig. 3, lane G).

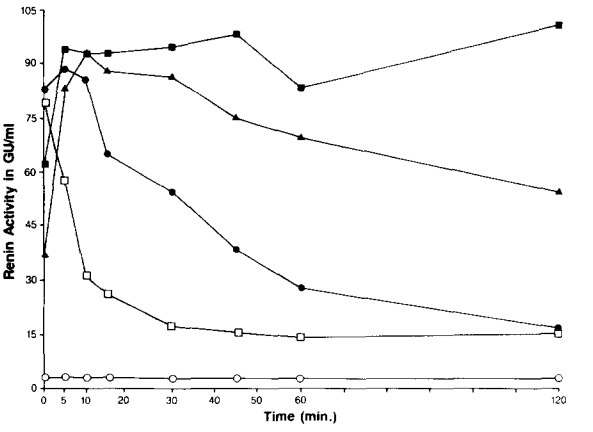

Fig. 2. Renin activity determined after trypsin activation of prorenin to the active enzyme at 37 °C. ○, 0 μg/ml; ■, 4 μg/ml; ▲, 10 μg/ml; ●, 25 μg/ml; □, 100 μg/ml. For assay conditions see text.

Amino acid composition

The composition of chorionic renin after acid hydrolysis has been determined. Our experimental determination (Table II) compares well with the composition inferred from the cDNA sequence

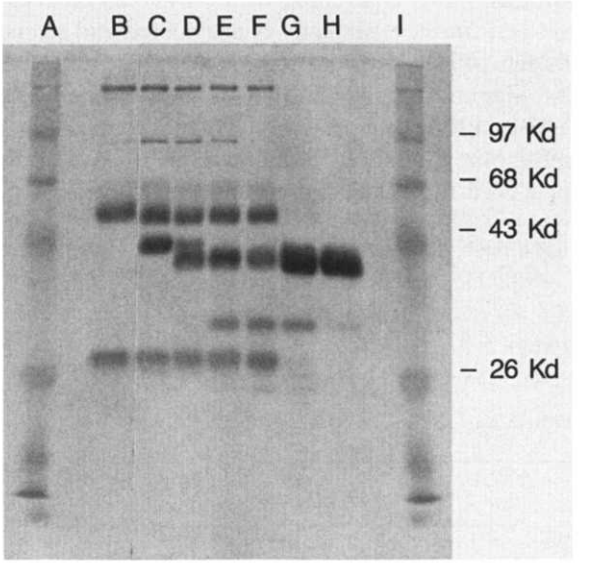

Fig. 3. Western blot analysis of the conversion of prorenin to renin using trypsin. Lanes A and I, molecular weight standards; lane B, antibody cross-reactivity; lanes C–F, trypsin activation of prorenin at time 0, 0.5, 30, and 120 min, respectively; lane G, post-affinity column; lane H, post-AcA-44 column. For assay conditions see text.

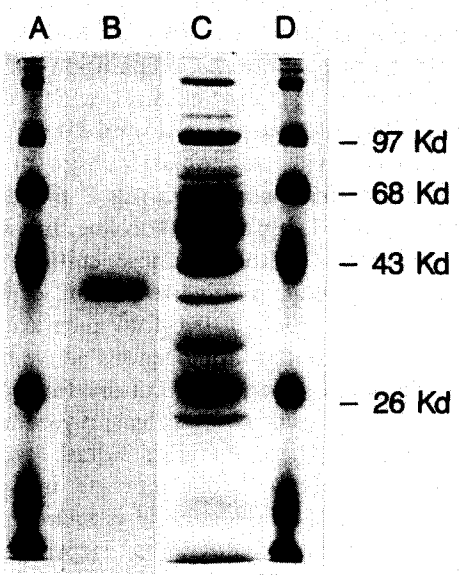

Fig. 4. SDS-PAGE of purified renin post-AcA-44 column (lane B) and pre-affinity column (lane C) under reducing conditions. Lanes A and D are molecular weight standards.

TABLE II

AMINO ACID COMPOSITION OF HUMAN CHORIONIC RENIN

Amino acid analysis was performed after acid hydrolysis of 1.1 μg purified chorionic renin.

	pmol	Arg Ref.	His Ref.	Theoretical
Asp	1085	27.0	27.6	29
Thr	1059	26.3	26.9	30
Ser	1149	28.6	29.2	33
Glu	1282	31.9	32.6	28
Pro	566	14.1	14.4	13
Gly	1555	37.8	39.5	34
Ala	699	17.4	17.8	16
Cys	0	0	0	0
Val	847	21.1	21.5	23
Met	226	5.6	5.7	8
Ile	777	19.3	19.8	22
Leu	1133	28.2	28.8	28
Tyr	615	15.3	15.6	17
Phe	708	17.6	18.0	19
His	236	5.9	6	6
Lys	583	14.5	14.8	15
Trp	0	0	0	3
Arg	402	10	10.2	10

published by Imai et al. [30] and that recently determined for purified human kidney renin [11].

N-terminal amino acid sequence

The chorionic renin has been subjected to sequential amino acid analysis through 27 amino acid residues. The sequence obtained (Fig. 5) is identical to that inferred from the cDNA sequence with the exception of the fifth residue, an asparagine residue which is a probable site of N-linked glycosylation. The sequence reported here is also identical to the sequence recently reported in a preparation of human kidney renin by Do et al. [11] with the exception of other probable sites of glycosylation. Renin derived from transfection of Chinese Hamster Ovary cells with cDNA for human preprorenin has been reported [19] to have the same amino terminal sequence as the renin that we obtain from chorionic cell culture.

Electrofocusing analysis

Upon subjecting the purified renin to IEF, five to seven active species can be separated. Fig. 6, lane B shows the comparison of the staining pattern of renin versus known isoelectric point standards. A duplicate unstained gel was cut into 96 slices and extracted in buffer C with 1 mg/ml bovine serum albumin for 6 hours at 4°C. After the extraction procedure, the enzyme activity was determined. As can be seen in Fig. 7, there are three major peaks of activity and other minor ones which agree with the staining pattern shown in Fig. 6. The same staining pattern was also observed on two-dimensional Western blots (IEF and SDS-PAGE) of tissue culture media containing prorenin, implying that the microheterogeneity is an artifact of neither the purification scheme nor the trypsin activation step.

As can be seen in Fig. 6, treatment of the active enzyme with neuraminidase decreases the microheterogeneity and shifts the protein bands to more basic species. Endoglycosidase D has no effect, while endoglycosidase F and endoglycosidase H apparently cause an increase in the microheterogeneity of the renin. The results seen using neuraminidase are probably due to a decrease in the

Leu—Thr—Leu—Gly— X —Thr—Thr—Ser—Ser—Val—Ile—Leu—Thr—Asn—Tyr— Met—Asp—Thr—Gln—Tyr—Tyr—Gly—Glu—Ile—Gly—Ile—Gly
5 10 15 20 25

Fig. 5. Amino-terminal sequence of human chorionic renin. Residue 5 is glycosylated asparagine from cDNA analysis.

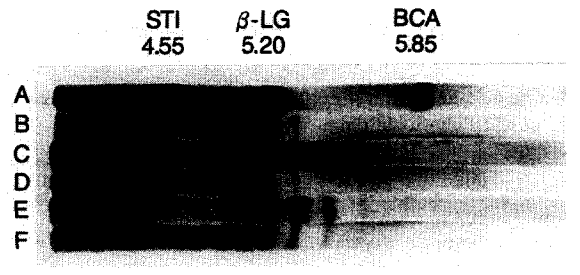

Fig. 6. Electrofocusing analysis of purified renin. Lane A, IEF standards; lane B, purified renin; lane C, purified renin coincubated with neuraminidase (5 min); lanes D–F, purified renin coincubated with endoglycosidase D, endoglycosidase F, and endoglycosidase H, respectively (120 min). STI, soybean trypsin inhibitor; β-LG, β-lactoglobulin; BCA, bovine carbonic anhydrase.

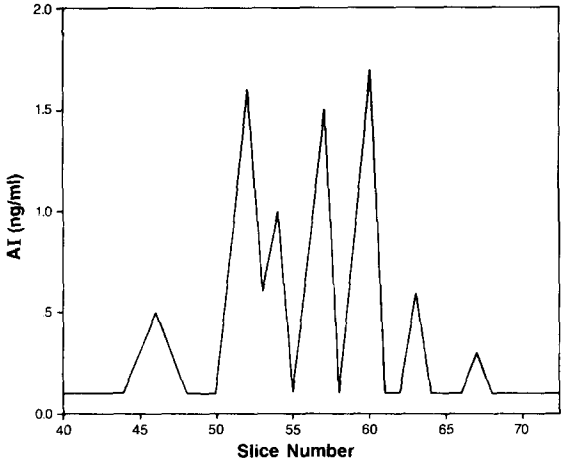

Fig. 7. Enzyme activity determined after subjecting the purified renin to IEF (duplicate of lane B, Fig. 6). AI, angiotensin I.

number of sialic acid residues covalently linked to the carbohydrate core of the protein.

Kinetic determination

Using pure human angiotensinogen from human plasma, the Michaelis complex of renin and its natural substrate has been determined to be 0.77 μM (Lineweaver-Burk analysis). The turnover number at saturating substrate was calculated as 1.3 s^{-1} in maleate buffer at pH 6.0 at 37 °C.

Discussion

The methods reported in this paper produce prorenin containing culture fluid with titers of 50–200 mGU/ml. Our rate of success in producing secretory cultures that generate high levels of prorenin from a chorionic preparation has been greater than 60%; those cultures which do not produce titers of over 50 mGU/ml after 2 weeks are discarded. The trophoblast culture produces maximal prorenin activity during 2–6 weeks of culture, followed by a slow decline for several weeks thereafter. Although these cultures are a rich source of renin relative to plasma or kidney homogenates, a 5000–10000-fold purification is still required to obtain homogenous active renin.

The decanted supernatant culture fluid can be stored frozen at −70 °C for at least a year without loss of activity. The ammonium sulfate fractionation affords only a 3-fold increase in specific activity but the 50-fold volume reduction makes the subsequent chromatographic steps practical. The QAE-Sephadex/cibracon blue column steps result in a preparation where prorenin consists of approximately 0.5–1% of the total protein.

Porcine pancreatic trypsin rapidly activates the pro-enzyme to active renin. The optimal concentration of trypsin (10 μg/ml) has been consistent throughout our work. Neither urinary nor plasma human kallikrein can activate chorionic prorenin under trypsin activation conditions, although combinations of acid/enzyme treatments for activation of amniotic fluid renin activity have not been done as described by Poisner et al. [31] and Hsueh et al. [32]. The present work has concentrated on the purification of active renin in milligram quantities. Further studies on the mechanism of activation are in progress.

Although the non-specific proteinase trypsin has been used for activation, and a number of possible sites of cleavage are possible from examination of the cDNA sequence [30] (e.g., Lys at -20, Arg at -18, Arg at -12), a single N-terminal sequence has been found. This implies that Lys-2–Arg-1 is a highly susceptible site for trypsin proteolysis. The small amount of over-proteolyzed material evident in the Western blot analysis is removed in subsequent chromatography steps.

The heterogeneity of renin by electrofocusing analysis is similar to that observed with the kidney enzyme [9,17]. A typical preparation of chorionic renin has three major active species with isoelectric points ranging from 4.8 to 5.2. Since these

species all have identical molecular weight on SDS-PAGE, variances in carbohydrate composition and particularly in sialic acid content is a probable cause of the heterogeneity. Our experiments with neuraminidase and endoglycosidase treatments under non-denaturing conditions support this point.

In conclusion, chorionic cell culture has provided the means for the routine purification of native human renin on a milligram scale. The properties of this renin appear to be similar to those reported for kidney-derived renin. The ability to produce milligram quantities of chorionic renin and prorenin will allow the study of both proteins and the activation pathway.

Acknowledgements

We are grateful to Dr. Herman Stein for the affinity column conditions, Mr. Anthony Fung for the affinity ligand, and Ms. Marcia Chekal for the production of human renin antibodies in rabbits. The composition and sequence analyses were carried out by Ms. Sarah Dorwin. The advice of Dr. Alan Poisner is gratefully acknowledged. We also thank Susan Clay, Cindy Davis, and Joan Doerrer for manuscript preparation.

References

1 Ganten, D., Unger, T. and Lang, R.E. (1984) Arzneim. Forsch. Drug Res. 34, 1391–1398.
2 Ondetti, M.A. and Cushman, D.W. (1984) CRC Crit. Rev. Biochem. 16, 381–411.
3 Hofbauer, K.G. and Wood, J.M. (1985) Trends Pharmacol. Sci. 6, 173–177.
4 Pals, D.T., Thaisrivongs, S., Lawson, J.A., Kati, W.M., Turner, S.R., DeGraaf, G.L., Harris, D.W. and Johnson, G.A. (1986) Hypertension 8, 1105–1112.
5 Smith, S.G., Seymour, A.A., Mazack, E.K., Boger, J. and Blaine, E.H. (1987) Hypertension 9, 150–156.
6 Szelke, M., Leckie, B., Hallett, A., Jones, D.M., Sueiras, J., Atrash, B. and Lever, A.F. (1982) Nature 299, 555–557.
7 Yokosawa, H., Holladay, L.A., Inagami, T., Haas, E. and Murakami, K. (1980) J. Biol. Chem. 255, 3498–3502.
8 Slater, E.E. and Strout, H.V. (1981) J. Biol. Chem. 256, 8164–8171.
9 Takahashi, S., Miura, R., Yutani, C. and Miyake, Y. (1986) J. Biochem. (Tokyo) 99, 307–310.
10 Shinagawa, T., Do, Y.-S., Tam, H. and Hsueh, W.A. (1986) Biochem. Biophys. Res. Commun. 139, 446–454.
11 Do, Y.-S., Shinagawa, T., Tam, H., Inagami, T. and Hsueh, W.A. (1987) J. Biol. Chem. 262, 1037–1043.
12 Atlas, S.A., Sealey, J.E., Hesson, T.E., Kaplan, A.P., Menard, J., Corvol, P. and Laragh, J.H. (1982) Hypertension 4 (Suppl. II), II-86–II-95.
13 Dzau, V.J. and Sands, K.E. (1982) Clin. Sci. 63, 163s–166s.
14 Johnson, R.L., Fleming, N.W. and Poisner, A.M. (1979) Biochem. Pharmacol. 28, 2597–2600.
15 Poisner, A.M., Wood, G.W., Poisner, R. and Inagami, T. (1982) Proc. Soc. Exp. Biol. Med. 169, 4–6.
16 Johnson, R.L., Poisner, A.M. and Crist, R.D. (1979a) Biochem. Pharmacol. 28, 1791–1799.
17 Galen, F.-X., Devaux, C., Guyenne, T., Menard, J. and Corvol, P. (1979) J. Biol. Chem. 254, 4848–4855.
18 Marcotte, P. and Egan, D. (1985) Fed. Proc. 44, 1568.
19 Poorman, R.A., Palermo, D.P., Post, L.F., Murakami, K., Kinner, J.H., Smith, C.W., Reardon, I. and Heinrikson (1986) Proteins Struct. Function Genet. 1, 139–145.
20 Poisner, A.M., Cheng, H.-C., Wood, G.W. and Poisner, R. (1983) Trophoblast Res. 1, 279–288.
21 Laemmli, U.K. (1970) Nature 227, 680–685.
22 Righetti, P.G. and Drysdale, J.W. (1974) J. Chromatogr. 98, 271–321.
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
24 Dorer, F.E., Lentz, K.E., Kahn, J.R., Levine, M. and Skeggs, L.T. (1978) Anal. Biochem. 87, 11–18.
25 Tewksbury, D.A., Dart, R.A. and Travis, J. (1981) Biochem. Biophys. Res. Commun. 99, 1311–1315.
26 Haber, E., Koerner, T., Page, L.B., Kliman, B. and Purnode (1969) J. Clin. Endocrinol. Metab. 29, 1349–1355.
27 Stein, H., Fung, A. and Cohen, J. (1985) Fed. Proc. 44, 1363.
28 Hsueh, W.A., Carlson, E.J. and Dzau, V.J. (1983) J. Clin. Invest. 71, 506–517.
29 Takii, Y. and Inagami, T. (1982) Biochem. Biophys. Res. Commun. 104, 133–140.
30 Imai, T., Miyazaki, H., Hirose, S., Hori, H., Hayashi, T., Kageyama, R., Ohkubo, H., Nakanishi, S. and Murakami, K. (1983) Proc. Natl. Acad. Sci. USA 80, 7405–7409.
31 Poisner, A.M., Johnson, R.L., Hanna, G. and Poisner, R.B. (1981) in Heterogeneity of Renin and Renin-Substrate: Proceedings of the 7th Meeting of the International Society of Hypertension (Sambhi, M.P., ed.), pp. 335–347, Elsevier/North-Holland, Amsterdam.
32 Hsueh, W.A., Carlson, E.J. and Israel-Hagman, M. (1981) Hypertension 3 (Suppl. I), I-22–I-29.