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Characterization of human prorenin expressed in mammalian cells from cloned cDNA

(renin/mammalian expression/hypertension)

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ABSTRACT **Human preprorenin was synthesized in Chinese hamster ovary (CHO) cells transfected with an expression vector containing renin cDNA sequences. These cells secrete an inactive form of renin (EC 3.4.23.15) that can be activated by trypsin. This inactive renin is precipitable by antibody generated against purified human renal renin and also by antisera generated to a synthetic peptide derived from the amino acid sequence of the pro segment of preprorenin (anti-propeptide), indicating that the secreted inactive enzyme is a form of prorenin. Analysis of [^{35}S]methionine-labeled proteins immunoprecipitated from CHO cell conditioned culture medium indicates that prorenin is expressed in CHO cells as two distinct forms that differ in their degree of glycosylation. *In vitro* trypsin activation of prorenin cleaves approximately 4.5 kDa from the protein, rendering it unreactive with the anti-propeptide antiserum but still recognizable by anti-renal renin antibody. These results show directly that the prorenin expressed by CHO cells is an inactive enzyme that is activated by trypsin cleavage of the pro segment. The ability to express human renin in this form will allow for the purification of both active and inactive forms of the enzyme in quantities sufficient for detailed physiological and structural studies.**

The renin–angiotensin system plays a pivotal role in blood pressure homeostasis and in the regulation of extracellular fluid volume. Renin (EC 3.4.23.15) is an aspartyl protease that catalyzes the first and rate-limiting step in the sequence of reactions that leads to the production of the potent pressor substance angiotensin II (1). Renin is found in highest concentration in kidney (2) from which it is secreted into the bloodstream; however, the enzyme has also been detected in several extrarenal tissues (3). Additionally, human kidney, plasma, and amniotic fluid contain an inactive form of renin that can be activated by a variety of treatments, e.g., incubation with trypsin (4). Although the inactive form is usually the predominant species in human plasma, accounting for up to 90% of the total renin (4), the molecular identity of inactive renin has been a matter of considerable dispute. Many biochemical and physiological questions relating to renin and prorenin remain unanswered, in part, due to the difficulty in obtaining the necessary quantities of these proteins in pure form.

Amino acid (5) and cDNA (6) sequence analyses of mouse submaxillary gland renin show that this enzyme is synthesized as a precursor, containing a proposed 18-amino acid amino-terminal signal sequence and a 45-amino acid pro segment followed by the active enzyme. cDNA (7) and genomic DNA sequence analyses (8–10) of human renin suggest that the human enzyme is also synthesized as preprorenin [with a 20-amino acid signal sequence and 46-amino acid pro segment (43)]. It has often been suggested that plasma and tissue inactive renins may be prorenin (4, 11), and this view is supported by experiments (12–14) showing that inactive renin from human plasma and kidney binds to antibodies raised against synthetic peptides corresponding to portions of the predicted pro segment of human prorenin (7).

To examine directly the properties of prorenin as well as to investigate its posttranslational processing, we have constructed a plasmid designed to express human preprorenin in mammalian cells. We report here that Chinese hamster ovary (CHO) cells transfected with this expression plasmid secrete an inactive form of renin that can be activated *in vitro* by trypsin under the same conditions that activate human inactive renins. Furthermore, we demonstrate that this expressed proenzyme is produced in two distinct glycosylated forms but that the presence of covalently bound sugar is not necessary for renin enzymatic activity. Mammalian cell expression methods should ultimately allow for the purification of sufficient quantities of renin and prorenin for biochemical and physiological studies.

MATERIALS AND METHODS

cDNA Cloning. Poly(A)$^+$ RNA was isolated from human kidney. An oligo(dT)-primed cDNA library was constructed from this RNA by synthesizing double-stranded cDNA (15) that was cloned into the *Eco*RI site of the bacteriophage vector λgt10 (16) using *Eco*RI linkers. The library was screened with probes derived from the human renin gene (8), and hybridizing cDNA inserts were sequenced (17). These inserts were comprised of human renin cDNA extending from base 8 of the protein coding region of the published sequence of Imai *et al.* (7) through the poly(A) tail. The sequence was identical to that presented by Imai *et al.* (7) with the exception of an adenine to cytosine transversion at base 204.

Construction of a Preprorenin Expression Plasmid. Eukaryotic expression vector pMTpro (obtained from K. Talmadge, California Biotechnology) consists of a 870-base-pair *Hin*dIII–*Bam*HI fragment from the human metallothionein II (MT-II) gene (18, 19) inserted into the polylinker of pUC9. This genomic fragment contains metal regulatory regions, glucocorticoid binding sites, and the promoter, transcription start, and initiator methionine of the metallothionein II gene (18). pMTpro DNA was digested with *Bam*HI and *Eco*RI and ligated to an *Eco*RI fragment comprised of the renin cDNA flanked by linkers. The 5′ tails of the noncohesive *Bam*HI and *Eco*RI termini were filled in using Klenow fragment of DNA polymerase and a mixture of deoxynucleoside triphosphates. This plasmid was circularized and used to transform *Esche-*

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Abbreviations: endo F, endoglycosidase F; CHO-CM, CHO cell conditioned medium.

richia coli MC1061. Plasmids containing renin cDNA inserts were isolated and characterized by restriction mapping and DNA sequencing. The resulting renin expression vector, pHR14, codes for a preprorenin molecule with a slightly altered signal sequence (see Fig. 1). The coding region is followed by the 3'-untranslated region including the polyadenylylation signal and a stretch of approximately 150 bases of poly(A). Plasmid pHR14 was cotransfected into CHO cells with pSV2neo (20) by the calcium phosphate precipitation method (21). Aminoglycoside-resistant cells were selected by growth in G418 at 400 μg/ml (GIBCO). Control cell pools were obtained by transfecting CHO cells with the parent expression vector, pMTpro or a closely related vector lacking the initiator methionine, pMT401.

Cell Culture. CHO cells were grown in a mixture of Dulbecco's modified Eagle's medium (DMEM) and Coon's F12 (Irvine Scientific) (1:1; vol/vol) supplemented with 10% (vol/vol) fetal calf serum, and penicillin at 50 units/ml, streptomycin at 50 μg/ml (GIBCO) in humid 5% CO_2/95% air at 37°C. For radiolabeling, cells were washed with Dulbecco's PBS and then incubated for 12–18 hr in DMEM/Coon's F12 containing one-fifth of the standard concentration of methionine plus 10% (vol/vol) dialyzed fetal calf serum and containing [^{35}S]methionine at 0.5 mCi/ml (~1000 Ci/mol; 1 Ci = 37 GBq; New England Nuclear). (For some experiments, increased transcription of the metallothionein promoter was induced with 1–10 × 10^{-5} M $ZnSO_4$.) In some experiments, tunicamycin at 2 μg/ml (Boehringer Mannheim) was added to the cultures 30 min prior to labeling and was also included at that concentration in the labeling cocktail.

Renin Assay. Inactive and active renin were assayed by the method of Atlas *et al.* (22). Inactive renin was activated with trypsin at 50–100 μg/ml in activation buffer [50 mM Tris·HCl (pH 8.0), 0.1 M NaCl, 3 mM EDTA, 10 mM benzamidine, and 0.5% bovine serum albumin (22)]. Renin activity was measured as the rate of angiotensin I production in the presence of 0.3 μM human angiotensinogen. Angiotensin I was quantitated by radioimmunoassay (Travenol-Genentech Diagnostics).

Production of Antibodies. Anti-renin antiserum (R1723), obtained from a rabbit immunized with pure human renal renin (23, 24), has a 50% inhibitory titer of 1:30,000 against 1 × 10^{-4} Goldblatt units of standard human renal renin (National Institute for Biological Standards and Control, London). The antiserum is specific for renin as demonstrated by immunodiffusion and immunoelectrophoresis analysis, and it does not crossreact with cathepsins D or B, renal kallikrein, or pepsin (24).

A rabbit antiserum (anti-propeptide) was generated against a 12-amino acid synthetic peptide corresponding to the carboxyl end of the putative pro segment of human prorenin (Arg-Leu-Gly-Pro-Glu-Trp-Ser-Gln-Pro-Met-Lys-Arg). The peptide, synthesized by solid-phase methods and purified (25, 26), was coupled to keyhole limpet hemocyanin and injected with adjuvants. The anti-propeptide antiserum had a titer of 1:6250 when tested against the synthetic propeptide in a solid-phase enzyme-linked immunosorbent assay and had undetectable binding to control peptides.

Immunoprecipitation of Expressed Activity. CHO cell conditioned culture medium (CHO-CM) (containing 3–30 × 10^{-5} Goldblatt units of renin) was incubated with anti-propeptide, anti-renin, or control nonimmune antisera (diluted 1:35 to 1:120) for 18 hr at 4°C after which an excess of protein A-Sepharose (Pharmacia) was added at room temperature for 1 hr. The protein A-Sepharose pellets were removed by centrifugation, and the supernatants were activated with trypsin and then assayed for renin activity. Separate aliquots of CHO-CM, diluted in activation buffer (22), were treated with trypsin (50–100 μg/ml) for 1 hr at room temperature and soybean trypsin inhibitor (100 μg/ml) for 10 min before immunoprecipitation.

Immunoprecipitation of Radiolabeled CHO-CM. S^{35}-labeled CHO-CM was diluted 1:1 in activation buffer containing 0.05% Nonidet P-40 and 0.05% $NaDodSO_4$. Antiserum was added to a final dilution of 1:300 and incubated at 4°C for 3–18 hr. Immunoglobulin was precipitated with an excess of protein A-Sepharose (Pharmacia). Pellets were washed three times with 50 mM Tris·HCl (pH 7.5), 0.15 M NaCl, 5 mM EDTA, 0.5% Nonidet P-40, ovalbumin at 1 mg/ml and once with the same buffer lacking ovalbumin. The final pellet was resuspended in gel sample buffer, electrophoresed through a $NaDodSO_4$/10% polyacrylamide gel (27), and fluorographed with Enlightning (New England Nuclear).

Endoglycosidase F Digestion. ^{35}S-labeled CHO-CM was adjusted with sodium phosphate, pH 6.5 (10 mM, final concentration) and EDTA (20 mM, final concentration) and incubated with endoglycosidase F (endo F) at 4 units/ml (New England Nuclear) for 12–18 hr at 37°C.

RESULTS

Renin Activity in Transfected CHO-CM. The expression plasmid pHR14 directs the synthesis of human preprorenin. This plasmid encodes a renin signal sequence modified at its amino terminus as compared to the natural signal sequence of human preprorenin; the putative signal peptidase cleavage site between amino acids 23 and 24, however, remains unchanged (Fig. 1). CHO-CM from confluent cultures of CHO cells transfected with either pHR14 or a control plasmid was assayed for renin activity with or without trypsin activation using human angiotensinogen as substrate (Table 1). Without trypsin activation, CHO-CM from pools of pHR14-transfected cells does contain detectable renin activity. However, following limited proteolysis with trypsin, under conditions that are known to activate human inactive renin (22), renin activity in the CHO-CM increases over

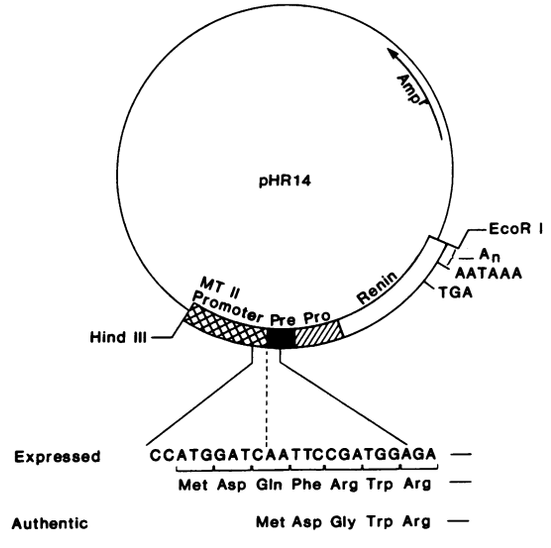

FIG. 1. Diagram of the preprorenin expression plasmid pHR14. The cDNA coding for the signal sequence ("pre"), the propeptide ("pro") and mature renin ("renin") was cloned into plasmid pMTpro that contains the regulatory elements and the initiator methionine from the human metallothionein II gene. The cDNA contained a translation termination site (TGA), polyadenylylation signal (AATAAA) and poly(A) tail (A_n). As shown, the construction codes for a signal sequence ("expressed") that differs from the authentic renin signal sequence ("authentic") at its amino-terminus (7). The dotted line indicates the location of the blunt ligation between the promoter fragment and the 5' end of the cDNA.

Table 1. Renin activity and trypsin-activated renin activity in supernatants from transfected CHO cells

Transfected plasmid	Trypsin	Renin activity, ng of AI/ml per hr	Relative renin activity
pHR14	−	23.4 ± 0.8	1
pHR14	+	664 ± 37	28
Control	−	<2	<0.1
Control	+	<2	<0.1

One Goldblatt unit is equivalent to 30,000 ng of angiotensin I (AI) per hr under the conditions of this experiment.

25-fold. Control supernatants do not contain detectable renin activity either before or after trypsin activation.

The expressed renin activity was further characterized by immunoprecipitation (Fig. 2). Trypsin-activatable renin activity in CHO-CM is immunoprecipitable by both anti-propeptide and anti-renin antisera (Fig. 2, *Left*) demonstrating that the enzyme is indeed a form of prorenin. If however, supernatants are treated with trypsin before antibody addition, only anti-renin but not anti-propeptide antiserum precipitates enzyme activity (Fig. 2, *Right*). This demonstrates that trypsin activation of expressed prorenin generates active renin by cleavage of the propeptide.

To further compare the expressed renin activity to that of authentic renal renin, the relative sensitivity of these two renins to a renin inhibitor was assayed. Accordingly, we synthesized and purified (25, 26) renin inhibitory peptide Gly-Gly-His-Pro-Phe-His-Statine-Ile-Phe-NH_2, a modification of a peptide described by Boger *et al.* (28). Fig. 3 shows that this peptide inhibits recombinant renin activity with a dose–response relationship identical to that observed with human kidney renin.

These results indicate that CHO cells transfected with the expression plasmid pHR14 secrete inactive prorenin, which is activated by trypsin cleavage, yielding a protein with the immunological and enzymatic properties of active human kidney renin.

Immunoprecipitation of Biosynthetically Labeled Forms of Expressed Renin. To biochemically characterize the prorenin secreted by CHO cells, cell protein was biosynthetically labeled with [^{35}S]methionine and secreted protein was analyzed, with or without trypsin activation, by immunoprecipitation, NaDodSO$_4$/PAGE and autoradiography. The resulting autoradiograms (Fig. 4) demonstrate that CHO-CM from cells transfected with pHR14 contain two proteins of molecular sizes 50.2 ± 0.7 and 48.9 ± 0.7 kDa (n = 8), which are immunoprecipitable with either anti-propeptide or anti-renin antisera (Fig. 4, lanes 4 and 5). Immunoprecipitation of trypsin-activated supernatants with anti-renin antiserum reveals two smaller proteins, 45.7 ± 0.6 and 44.2 ± 0.3 kDa (n = 3) (Fig. 4, lane 6). Neither of these lower molecular size forms is precipitable by anti-propeptide antiserum (data not shown). Both the shift in size of the immunoprecipitable protein upon trypsin treatment and the loss of anti-propeptide reactivity are consistent with the complete removal of the putative 43-amino acid propeptide. The immunoprecipitable

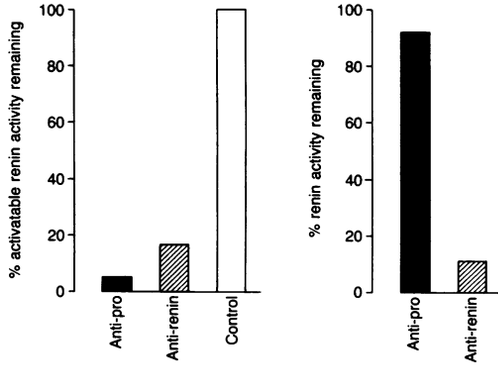

FIG. 2. Immunoprecipitation of expressed prorenin and trypsin-activated prorenin. (*Left*) CHO-CM containing prorenin equivalent to 3 × 10^{-4} Goldblatt units was incubated with anti-propeptide antiserum (anti-pro), anti-human renin antiserum (anti-renin), or control antiserum (control). Following addition of protein A-Sepharose and centrifugation, nonbound material was trypsin activated and renin activity was measured. (*Right*) CHO-CM containing 3 × 10^{-5} Goldblatt units of renin was first trypsin activated and then incubated with antisera and protein A-Sepharose as described above. Renin activity remaining in the supernatant was measured directly.

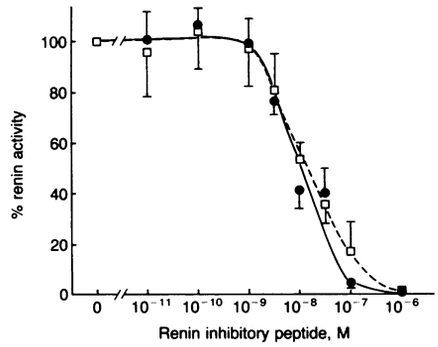

FIG. 3. Inhibition of recombinant renin and human kidney renin by a renin inhibitory peptide. Human kidney renin (□) and CHO-CM activated with trypsin (●) (3–10 × 10^{-5} Goldblatt units in 100 μl) were incubated for 1 hr at room temperature with various concentrations of a statine-containing renin inhibitory peptide. Renin activity in samples was then assayed and plotted as percent of the activity measured in the absence of inhibitor. Data from several experiments were pooled to generate the figure.

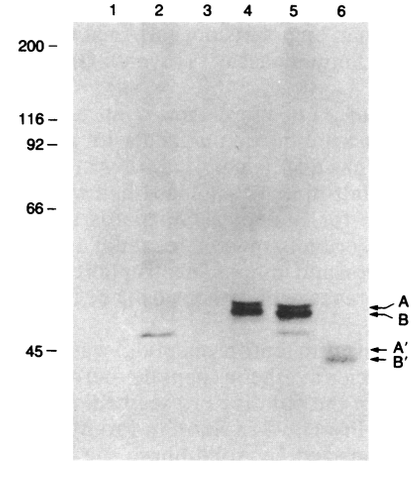

FIG. 4. Immunoprecipitation of prorenin and active renin from [^{35}S]methionine-labeled CHO-CM. CHO cells transfected with the preprorenin construct pHR14 (lanes 4–6) or control plasmid pMT401 (lanes 1–3) were labeled with [^{35}S]methionine and secreted protein was incubated with anti-propeptide antiserum (lanes 1 and 4) or anti-human renin antiserum (lanes 2 and 5). Other supernatants were treated with trypsin at 100 μg/ml followed by soybean trypsin inhibitor (lanes 3 and 6) before immunoprecipitation with anti-renin antiserum. Both anti-propeptide and anti-renin antisera specifically immunoprecipitate two proteins (lanes 4 and 5, arrows A and B) from pHR14-transfected CHO-CM. If the pHR14-transfected CHO-CM was treated with trypsin before immunoprecipitation, anti-renin antibody precipitates two proteins of lower molecular size (lane 6, arrows A′ and B′). The band in lane 5 that migrates between arrows B and A′ is nonspecific, since it also appears in the immunoprecipitate from a control culture (lane 2). Molecular size standards in kDa are shown on the left.

proteins are not observed in immune precipitates from cells transfected with a control plasmid (pMT401) (Fig. 4, lanes 1–3).

To investigate the possibility that the two distinct prorenin forms represent heterogeneous glycosylation, proteins immunoprecipitated from endo F-treated CHO-CM were compared to those immunoprecipitated from untreated CHO-CM. Endo F cleaves both complex and high-mannose N-linked sugar chains leaving a single *N*-acetylglucosamine moiety attached to each glycosylated asparagine residue (29). Whereas anti-propeptide antiserum immunoprecipitates two distinct protein species from untreated CHO-CM (Fig. 4, lane 4 and Fig. 5, lane 1), endo F treatment results in only a single immunoprecipitable protein (Fig. 5, lane 3). This protein migrates on $NaDodSO_4$/polyacrylamide gels at a molecular size of 44 kDa ($n = 2$) and has an identical mobility to prorenin expressed in bacteria (Fig. 5, lane 5), which is not posttranslationally modified by glycosylation. (The expression of prorenin and renin in bacterial cells will be described elsewhere.) The specificity of the immune precipitations is shown by controls using preimmune serum (Fig. 5, lanes 2 and 4). The expression of a unique prorenin polypeptide that exists in two glycosylated forms was further demonstrated by immunoprecipitation experiments using radiolabeled cultures grown in the presence of tunicamycin. Tunicamycin treatment, which inhibits the transfer of core sugars to peptide chains (30), also results in the appearance of a 44-kDa form of prorenin precipitable with anti-propeptide antiserum (data not shown). Interestingly, deglycosylation has no apparent effect on enzymatic activity. When samples of activated prorenin (5,000–10,000 ng of angiotensin per hr) were treated with an excess of endo F, the deglycosylated material had an activity 106 ± 34% of the untreated glycosylated material (100 ± 21%) ($n = 6$).

DISCUSSION

We have constructed an expression vector that directs the synthesis of human preprorenin upon transfection into CHO cells. Our results show that these cells secrete an inactive form of renin that can be activated by trypsin *in vitro* under the same conditions that activate natural inactive renins. This trypsin-activatable renin activity is immunoprecipitable by antibodies directed against either a synthetic peptide derived from the putative carboxyl terminus of the pro segment or by antibodies generated against purified human kidney renin. Furthermore, immunoprecipitation of [^{35}S]methionine-labeled CHO-CM shows that the size of the secreted enzyme is consistent with the secretion of a prorenin molecule. These immunochemical properties indicate that the secreted protein is indeed a form of prorenin although an unambiguous identification of the amino terminus of the secreted product must await its purification and sequencing.

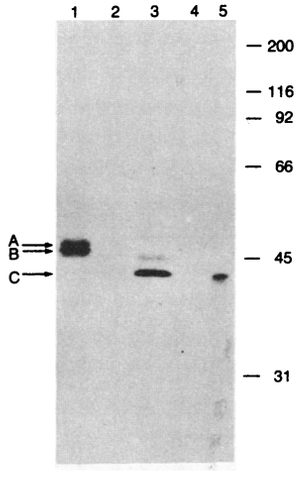

FIG. 5. Effect of endo F on prorenin secreted by CHO cells. Cultures of cells transfected with pHR14 were labeled with [^{35}S]methionine and CHO-CM was incubated with (lanes 3 and 4) or without (lanes 1 and 2) endo F at 4 units/ml. Prorenin was then immunoprecipitated with anti-propeptide (lanes 1 and 3) or preimmune (lanes 2 and 4) antisera and analyzed on $NaDodSO_4$/10% polyacrylamide gels. Anti-propeptide antiserum immunoprecipitates two proteins from untreated supernatants (lane 1, arrows A and B) and one protein of lower molecular size from endo F-treated CHO-CM (lane 3, arrow C). Protein produced by bacteria containing an expression plasmid for methionylprorenin (unpublished data) is also immunoprecipitated with anti-propeptide antisera (lane 5). Molecular size standards in kDa are shown on the right.

It has been reported that kidney and plasma inactive renins are recognized by antibodies directed against prosegment synthetic peptides (12–14), providing strong evidence that these inactive renins are prorenin. Our result that expressed prorenin is an inactive but activatable form of renin supports this hypothesis.

In prorenin-containing CHO-CM we do detect a low level of renin activity in the absence of added trypsin (Table 1). This may be due in part to a slow rate of proteolytic activation of prorenin resulting from the presence in the medium of proteases that are normally secreted by CHO cells. In accord with this idea, the percentage of enzyme that is active in the absence of added trypsin increases with time upon storage of the supernatants at 4°C (unpublished observations). This spontaneous activation correlates with the appearance of immunoprecipitable renin proteins that are intermediate in size between prorenin and trypsin-activated renin as measured by gel electrophoresis.

Following trypsin activation, the expressed renin activity is inhibitable by a statine-containing renin inhibitory peptide. The concentration dependence of this inhibitor is identical to that observed when it is tested against purified human renal renin (Fig. 3), suggesting that the authentic and expressed enzymes are very similar. Furthermore, the reduction in apparent molecular size of approximately 4.5 kDa following trypsin activation (Fig. 4) and the fact that the anti-propeptide antiserum does not recognize the activated enzyme (Fig. 2) both argue that trypsin cleaves off the prosegment at the predicted site for *in vivo* processing, leucine-67 (7). This has now been directly confirmed by purification and amino-terminal sequencing of the trypsin-treated active (400–800 Goldblatt units/mg) enzyme (details of which will be published elsewhere).

Immunoprecipitation of [^{35}S]methionine-labeled CHO-CM shows that prorenin is synthesized in CHO cells as two distinct forms having molecular sizes of 50.2 and 48.9 kDa as determined by $NaDodSO_4$/PAGE, consistent with values reported by others for human inactive renins. Using $NaDodSO_4$/polyacrylamide gels for size determinations, McIntyre *et al.* (31) described a 48-kDa inactive renin from human kidney, and Acker *et al.* (32) immunoprecipitated a 54-kDa inactive form from cultured chorionic cells. Molecular sizes derived from gel filtration of inactive renins from human kidney and plasma have been reported in the range of 51 to 60 kDa (4, 31, 33, 34).

Our results using endo F (Fig. 5) demonstrate that the different forms of prorenin secreted by CHO cells reflect differences in glycosylation and that as estimated by mobility on $NaDodSO_4$/polyacrylamide gels, N-linked sugars account for 5–6 kDa of the molecular size of the expressed proenzyme. It is possible that heterogeneous glycosylation of prorenin *in vivo* could account in part for some unresolved observations regarding the relationships among the various human renin forms. For example, it has been claimed that trypsin-activated plasma inactive renin is larger than active plasma renin (4, 34). One possible explanation is that secreted

inactive prorenin is more heavily glycosylated than secreted active renin. Furthermore, it has been reported that, in contrast to renal and plasma renins, bovine pituitary renin does not bind to Con A (35) suggesting a difference in the glycosylation of the enzyme in the pituitary. We have shown here that removal of sugar residues does not alter human renin enzymatic activity; however, other properties such as the rate or mode of secretion from cells or plasma clearance rate could be a function of glycosylation.

It is of interest that CHO cells secrete predominantly, if not exclusively, prorenin and not mature renin. Cleavage of the propeptide may require specific protein processing systems that may not be present in CHO cells. Experiments with proinsulin have shown that its processing to mature insulin occurs in conjunction with its being packaged into granules and that this process occurs only in differentiated secretory cells and not in L cells or COS cells (36–38). It has been reported however, that COS cells can proteolytically process preprosomatostatin to somatostatin (39).

The ability to produce human renin and prorenin by recombinant DNA techniques will now permit both a more detailed investigation of their physiological roles as well as the isolation of large amounts of enzyme for x-ray crystallographic analysis. Current three-dimensional models of human renin are based on x-ray data derived from other aspartyl proteases, together with the primary sequence of human renin (40–42). It will be possible to build more accurate models, useful for the rational design of renin inhibitors, from crystallographic data derived directly from the human enzyme and proenzyme.

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1. Skeggs, L. T., Dorer, F. E., Levine, M., Lentz, K. E. & Kahn, J. R. (1980) *Adv. Exp. Med. Biol.* **130**, 1–23.
2. Inagami, T. (1981) in *Biochemical Regulation of Blood Pressure*, ed. Soffer, R. L. (Wiley, New York), pp. 39–73.
3. Debuben, A., Inagami, T. & Ganten, D. (1983) in *Hypertension*, eds. Genest, J., Kuchel, O., Hamet, P. & Cantin, M. (McGraw-Hill, New York), 2nd Ed., pp. 194–209.
4. Sealey, J. E., Atlas, S. A. & Laragh, J. H. (1980) *Endocr. Rev.* **1**, 365–391.
5. Misono, K. S., Chang, J. J. & Inagami, T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4858–4862.
6. Panthier, J. J., Foote, S., Chambraud, B., Strosberg, A. D., Corvol, P. & Rougeon, F. (1982) *Nature (London)* **298**, 90–92.
7. 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.
8. Hardman, J. A., Hort, Y. J., Catanzaro, D. F., Tellam, J. T., Baxter, J. D., Morris, B. J. & Shine, J. (1984) *DNA* **3**, 457–468.
9. Hobart, P. M., Fugliano, M., O'Connor, B. A., Schaefer, I. M. & Chirgwin, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5026–5030.
10. Miyazaki, H., Fukamizu, A., Hirose, S., Hayashi, T., Hori, H., Ohkubo, H., Nakanishi, S. & Murakami, K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5999–6003.
11. Leckie, B. J. (1981) *Clin. Sci.* **60**, 119–130.
12. Bouhnik, J., Fehrentz, J. A., Galen, F. X., Seyer, R., Evin, G., Castro, B., Menard, J. & Corvol, P. (1985) *J. Clin. Endocrinol. Metab.* **60**, 399–401.
13. Kim, S. J., Hirose, S., Miyazaki, H., Veno, N., Higashimori, K., Morinaga, S., Kimura, T., Sakakibara, S. & Murakami, K. (1985) *Biochem. Biophys. Res. Commun.* **126**, 641–645.
14. Atlas, S. A., Christofalo, P., Hesson, T., Sealey, J. E. & Fritz, L. C. (1985) *Biochem. Biophys. Res. Commun.* **132**, 1038–1045.
15. Fiddes, J. C. & Goodman, H. M. (1979) *Nature (London)* **281**, 351–356.
16. Huynh, T., Young, R. A. & Davis, R. W. (1985) in *DNA Cloning Techniques, A Practical Approach*, ed. Glover, D. M. (IRL, Oxford), pp. 49–78.
17. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
18. Karin, M. & Richards, R. I. (1982) *Nature (London)* **229**, 797–802.
19. Burgess, T. L., Craik, C. S. & Kelly, R. B. (1985) *J. Cell Biol.* **101**, 639–645.
20. Southern, P. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
21. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
22. Atlas, S. A., Hesson, T. E., Sealey, J. E., Pharmgrongartama, B., Laragh, J. H., Ruddy, M. C. & Purcell, M. (1984) *J. Clin. Invest.* **73**, 437–447.
23. Slater, E. E. & Strout, H. V. (1981) *J. Biol. Chem.* **256**, 8164–8171.
24. Hsueh, W. A., Carlson, E. J. & Dzau, V. J. (1983) *J. Clin. Invest.* **71**, 506–517.
25. Merrifield, R. B. (1964) *Biochemistry* **3**, 1385–1390.
26. Schenk, D. B., Johnson, L. K., Schwartz, K., Sista, H., Scarborough, R. M. & Lewicki, J. A. (1985) *Biochem. Biophys. Res. Comm.* **127**, 433–442.
27. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
28. Boger, J., Lohr, N. S., Ulm, E. H., Poe, M., Blaine, E. H., Fanelli, G. M., Lin, T. Y., Payne, L. S., Schorn, T. W., La Mont, B. I., Vassil, T. C., Stabilito, I. I., Veber, D. F., Rich, D. H. & Bopari, A. S. (1983) *Nature (London)* **303**, 81–84.
29. Elder, J. H. & Alexander, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4540–4544.
30. Duksin, D. & Mahoney, W. C. (1982) *J. Biol. Chem.* **257**, 3105–3109.
31. McIntyre, G. D., Pau, B., Hallett, A., Leckie, B. J. & Szelke, M. (1984) *J. Hypertension* **2**, 305–310.
32. Acker, G. M., Galen, F. X., Devaux, C., Foote, S., Papernik, E., Pesty, A., Menard, J. & Corvol, P. (1982) *J. Clin. Endocrinol. Metab.* **55**, 902–909.
33. Hsueh, W. A., Luetscher, J. A., Carlson, E. J. & Grislis, G. (1980) *Hypertension* **2**, 750–756.
34. Inagami, T., Okamoto, H., Ohtsuki, K., Shimamoto, K., Chao, J. & Margolius, H. S. (1982) *J. Clin. Endocrinol. Metab.* **55**, 619–627.
35. Hirose, S., Ohsawa, T., Murakami, K. & Inagami, T. (1982) *Exp. Brain Res.* Suppl., **4**, 53–63.
36. Moore, H.-p. H., Walker, M. D., Lee, F. & Kelly, R. B. (1983) *Cell* **35**, 531–538.
37. Lomedico, P. T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5798–5802.
38. Laub, O. & Rutter, W. J. (1983) *J. Biol. Chem.* **258**, 6043–6050.
39. Warren, T. G. & Shields, D. (1984) *Cell* **39**, 547–555.
40. Sibanda, B. L., Blundell, T., Hobart, P. M., Fogliano, M., Bindra, J. S., Dominy, B. W. & Chirgwin, J. M. (1984) *FEBS Lett.* **174**, 102–111.
41. Akahane, K., Umeyama, H., Nakagawa, S., Moriguchi, I., Hirose, S., Iizuka, K. & Murakami, K. (1985) *Hypertension* **7**, 3–12.
42. Carlson, W., Karplus, M. & Haber, E. (1985) *Hypertension* **7**, 13–26.
43. Hirose, S., Kim, S.-j., Miyazaki, H., Park, Y.-s. & Murakami, K. (1985) *J. Biol. Chem.* **260**, 16400–16405.