Characterization of Recombinant Human Prorenin and Renin

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SUMMARY A cell line that secretes substantial quantities of recombinant human prorenin was prepared by transfecting Chinese hamster ovary cells with a gene encoding prorenin. The prorenin was purified to homogeneity and was found to have a single amino terminus, reflecting cleavage after a typical 23 amino acid signal sequence. The purified inactive prorenin was not a substrate for active renin and was not capable of self-activation. Prorenin could be converted to renin by addition of exogenous protease, and deglycosylation of the prorenin did not alter the sensitivity to protease activation. The enzymatic activity of deglycosylated renin was kinetically identical to that of the native protein. Multimilligram quantities of recombinant human renin and prorenin were purified, providing suitable material for studies directed toward greater understanding of the function of these proteins and for structural studies such as x-ray diffraction for use in design of renin inhibitors. (Hypertension 11: 713–716, 1988)

KEY WORDS • renin • kinetic analysis • recombinant protein • enzyme activation • deglycosylation •

HUMAN renin and prorenin are present in extremely low amounts in the kidney and plasma and are easily altered by proteases present in these tissues. As a consequence, it has been difficult to obtain sufficient, stable quantities of the purified proteins to perform the types of studies required to fully understand their properties and to produce crystals suitable for structural studies. We have previously reported that Chinese hamster ovary cells transfected with a gene encoding human prorenin secrete prorenin into the culture media that is biochemically identical to kidney prorenin and can be converted to active renin by trypsin treatment.1,2 This report describes biochemical characterization of purified recombinant renin and prorenin and the effects of deglycosylation on their activities. We also show that, although prorenin can be converted to active renin by exogenous trypsin, purified recombinant prorenin is not a substrate for itself or for active renin.

Materials and Methods

Cell Culture

The development of Chinese hamster ovary cell lines transfected with a human prorenin–containing plasmid under the control of the metallothionein promoter that secrete prorenin has been described by Fritz et al.1 Cells were grown to confluency in roller bottles and then switched to serum-free medium containing activators of the metallothionein promoter1; prorenin-containing conditioned medium from the cells was collected and stored for further purification. Active renin was generated from the prorenin by addition of trypsin bound to Sepharose beads,3 which were removed by centrifugation before purification.

Renin Assay

Active and inactive renins were assayed by the method of Atlas et al.4 Activation of prorenin for assays was performed by addition of trypsin, 10 μg/ml. Activity was measured as the rate of angiotensin I produced in the presence of 0.2 μM purified human angiotensigen (produced by Duane Tewksbury; see Reference 5). Angiotensin I was quantitated by radioimmunoassay (Travenol-Genentech Diagnostics, Cambridge, MA, USA). In some experiments, in-
creased amounts of substrate were used and were compensated for by the addition of smaller volumes of reaction contents to the radioimmunoassay. Inhibition of renin activity by pepstatin was determined after preincubation of renin with varying concentrations of pepstatin in dimethyl sulfoxide (added to reaction buffer at a dilution of 1:100) for 30 minutes at room temperature before the addition of substrate.

**Endoglycosidase F Treatment**

Purified prorenin or renin was treated in 0.1 M sodium acetate, pH 6.5, with 4 units of endoglycosidase F (Endo F; New England Nuclear, Boston, MA, USA) per milligram of protein, for at least 5 days at 37°C to remove N-linked carbohydrate. Completeness of the reaction was assessed by a change in the migration of the products on sodium dodecyl sulfate gels (see the next section) or by the appearance of aspartic acid at Cycle 5 in N-terminal sequence analysis. Only those preparations in which greater than 90% of the Coomassie blue–staining material had shifted in migration were used for the kinetic studies.

**Gel Electrophoresis and Sequencing**

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to the method of Laemmli, with 12.5% acrylamide/0.8% bis-acrylamide. Sequencing was performed by automated Edman degradation using an Applied Biosystems gas phase microsequencer (Foster City, CA, USA).

**Results**

**Characterization After Purification**

As shown in Figure 1, sodium dodecyl sulfate–polyacrylamide gel electrophoresis of purified (C.T. Carilli, unpublished observations, 1987) recombinant human prorenin or trypsin-generated recombinant renin (Lanes 2 and 4 in Figure 1) demonstrated that greater than 95% of the protein migrates at molecular weights of approximately 47,000 and 42,000, respectively. In confirmation of previous results, some preparations of purified renin and prorenin migrated as two separate bands differing by an apparent molecular weight of about 2000. Removal of complex carbohydrate chains from renin by treatment with Endo F resulted in a single band with an apparent reduction in molecular weight of about 5000 to 7000 (see Figure 1, Lane 3). Similar results were obtained for prorenin (data not shown). These results suggest that the carbohydrate chains of recombinant prorenin and renin are heterogeneous.

N-Terminal sequence analyses of both purified preparations are consistent with a purity greater than 95%, as in both cases only a single sequence was detected. The amino terminus of the prorenin was identical to that found for recombinant prorenin expressed in murine cells (representing cleavage after a 23 amino acid signal peptide); that of the renin was the same as that found for human renal renin (representing cleavage after a 43 amino acid prosegment). The latter sequence showed no amino acid at Residue 5, consistent with the expected complex carbohydrate attachment site.

Purification of both prorenin and renin was required to stabilize these preparations against proteolytic degradation by proteases secreted by the Chinese hamster ovary cells, as this degradation (leading to both activation of prorenin and destruction of active renin) was seen to occur in partially purified preparations.

**Activation of Prorenin**

It was of interest to determine whether prorenin was capable of self-activation, in a manner analogous to the activation of pepsinogen to pepsin, since both are members of the aspartyl protease class of enzymes. As shown in Figure 2, however, purified prorenin did not self-activate, that is, there was no increase in the percentage of active renin present, even at the pH optimum for renin. Furthermore, prorenin is not a substrate for purified renin, since the addition of exogenous renin did not result in the cleavage of prorenin.

It is also shown in Figure 2 that removal of carbohydrate with Endo F treatment did not increase the percentage of active renin, demonstrating that this preparation of Endo F does not contain any contaminating proteases that are capable of activating prorenin. Also assessed was whether the carbohydrate chains themselves could affect the activation of prorenin. The results in Figure 3 demonstrate that removal of sugars from the prorenin does not change the profile of activation with trypsin (where increasing amounts of trypsin first generate active renin and then begin to inactivate the renin produced by further proteolysis at higher concentrations).

**Kinetic Characterization of Renin**

To determine whether the glycosylation of renin affects its activities, preparations with or without intact

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**Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of expressed renin and prorenin, stained with Coomassie blue.** Lane 1: total protein, 20 μg, in concentrated Chinese hamster ovary (CHO) cell supernatant (representing 0.5 ml of supernatant). Lanes 2 and 3: purified active renin, 20 μg, before and after treatment with endoglycosidase F (Endo F). Lane 4: purified prorenin, 20 μg. Lane 5: molecular weight (MW) markers.
carbohydrate chains were compared. Figures 4 and 5 display measurements of the $K_m$ for cleavage of purified human angiotensinogen and the $K_t$ for pepstatin inhibition of renin before and after treatment with endoglycosidase F (Endo F). From both figures it can be seen that removal of carbohydrate had essentially no effect on the active site structure.

Discussion
Recombinant human prorenin has been shown to be produced by Chinese hamster ovary cells transfected with the human preprorenin coding sequences. This protein can be purified to homogeneity or converted to active renin by trypsin treatment and subsequently purified as such. Both proteins have the N-termini expected from the complementary DNA sequence and seen in the corresponding proteins purified from natural sources.

The purified recombinant prorenin was shown to be incapable of self-activation or activation by purified renin. This result is expected in light of the strict substrate specificity of renin, but it is significant in that other aspartyl proteases have been shown to self-activate. One caveat, however, is that these studies were performed on recombinant material, which may not be identical to circulating inactive renin. Interestingly, the dose-response curve for activation by exogenously added trypsin was not shifted by removal of the carbohydrate residues from the prorenin by treatment with Endo F.

Active renin was also examined for effects of deglycosylation on enzymatic activity. Removal of the sugars had no apparent effect on the $K_m$ for substrate or the $K_t$ for pepstatin, suggesting that there is little change in the active site upon deglycosylation. It will be interesting to determine the effects of carbohydrate removal on the properties of renin and prorenin in vivo, for example, on metabolic stability or activation. It is of note that mouse submaxillary gland renin, which has identical enzymatic properties in vitro to human renal renin, is not glycosylated.

The purification schemes developed for both recombinant prorenin and renin have been amenable to scale-up, so that 100-mg quantities of the purified proteins are available (C.T. Carilli, unpublished observations, 1987). These preparations can be used for crystallization of the proteins, and analysis of diffraction-quality crystals is underway (M.N.G. James, unpublished observations, 1987).

These studies support the utility of the recombinant
DNA approach for gaining greater understanding of the biology of the renin-angiotensin system.

Acknowledgments

The authors are grateful for the tissue culture expertise of Jean Kloss, Cheryl Anderson, Carmen Bryant, Line Hawes, Connie Tam, and Yu Wang.

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Characterization of recombinant human prorenin and renin.
C T Carilli, J L Vigne, L C Wallace, L M Smith, M A Wong, J A Lewicki and J D Baxter

Hypertension. 1988;11:713-716
doi: 10.1161/01.HYP.11.6.713

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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