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Expression of a deletion mutant of the prosegment of human prorenin in Chinese hamster ovary cells

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Expression plasmids encoding native human preporenin and a mutant deleted in its entire prosegment were transfected into Chinese hamster ovary cells. The cells transfected with the expression plasmid of native preporenin secreted exclusively inactive prorenin, while the cells transfected with the mutant secreted the active enzyme. The secreted amount of renin from the latter cells was much lower than that of prorenin from the former ones, although these two enzymes had little difference in specific activity after trypsin activation. These results suggest that the prosegment plays an important role in the secretory process of renin, although the fully active enzyme can be formed in its absence.

Renin; Gene expression; Protein folding; (Chinese hamster ovary cell)

1. INTRODUCTION

The renin-angiotensin system plays an important role in the regulation of blood pressure and hydromineral balance. Renin (EC 3.4.23.15), an aspartyl protease, is the key enzyme in the system in which it controls the rate-limiting step in the formation of a potent vasoactive peptide, angiotensin II, by catalyzing the formation of AI from angiotensinogen [1]. Renin is synthesized initially as prorenin, an inactive precursor, in the juxtaglomerular cells of kidney. The precursor contains a 'prosegment', which is removed to produce mature, active renin by proteolytic cleavage during intracellular transport [2,3].

Such prosegments have been found in many secretory proteins although their lengths, locations in the precursor, and structures are different from one protein to another. There are several possible functions of a prosegment, such as acting as a linker peptide between the signal peptide and the mature protein to ensure the cleavage of the signal peptide [4,5], guiding correct folding of the protein [6], acting as a sorting signal through the secretory pathway [7,8], and providing a source of proenzyme for local activation like the blood clotting enzymes.

In the present study, to investigate the role of the prosegment of renin, native human prorenin and a mutant deleted in its entire N-terminal prosegment were expressed in CHO cells. The results suggest that the prosegment plays an important role in the secretory process of renin.

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Abbreviations: CHO cells, Chinese hamster ovary cells; AI, angiotensin I

2. MATERIALS AND METHODS

2.1. *Plasmid construction and DNA transfection*

Site-directed mutagenesis was performed as described by Morinaga et al. [9]. The oligonucleotide used to delete the sequence coding for the 43 amino acid N-terminal prosegment of human preprorenin is shown in fig.1. A mutant plasmid, pSVDΔPRn, was constructed by exchanging the mutated fragment with the corresponding fragment of pSVDPRnPA33, an expression plasmid of native human preprorenin [10]. The expression plasmids were transfected into CHO cells, which have a mutation in the dihydrofolate reductase gene, by calcium phosphate coprecipitation [11]. The transfected cells were cultured in Dulbecco's modified Eagle's medium containing 11.5 mg/l proline and 10% dialyzed fetal calf serum at 37°C in 5% CO_2.

2.2. *Assays*

Culture media were assayed for active renin and total renin (active renin, and prorenin after activation with 0.1 mg/ml trypsin) activities by an AI generating assay method as described previously [12]. Prorenin activity was deduced by taking the difference between these two measurements. Renin concentration in the medium was determined with a Renin RIA Pasteur kit (Diagonostic Pasteur, France). Specific activity was evaluated from the values of renin activity and renin content in the medium.

2.3. *Radiolabeling and immunoprecipitation*

Radiolabeling of prorenin and renin, and their immunological identification were performed as described previously [13]. Cells in 35 mm plates were incubated with 1 ml of methionine-free Dulbecco's modified Eagle's medium containing 0.4 mCi/ml of [^{35}S]methionine (> 1000 Ci/mmol; Amersham), 11.5 mg/l proline and 10% dialyzed fetal calf serum. The culture media were collected after 48 h of incubation, and then immunoprecipitated with anti-human renin antiserum and protein-A Sepharose. The immunoprecipitated proteins

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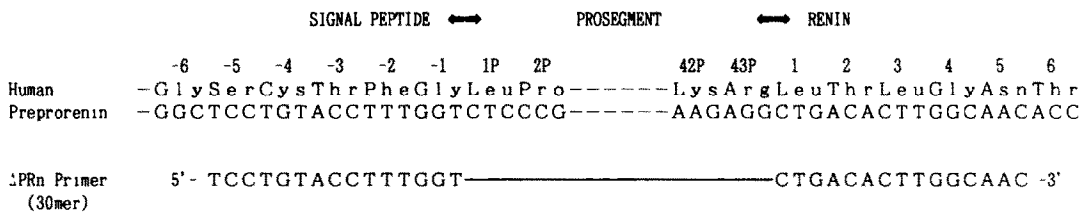

Fig.1. Nucleotide sequence of the pertinent region of human preprorenin cDNA and the mutagenic primer to delete the sequence coding for the entire 43 amino acid prosegment. The oligonucleotide sequence of the primer is shown below the sequence of preprorenin cDNA.

were analyzed by SDS-polyacrylamide gel electrophoresis followed by fluorography. The relative amounts of the immunoprecipitated proteins were determined by densitometric scanning.

2.4. *Northern blot analysis*

Total cellular RNA was isolated from the transfected cells by the method of Gough [14]. RNA was denatured with glyoxal, size-separated in 1.2% agarose gel, and blotted onto GeneScreen Plus membrane (New England Nuclear, Boston, MA). The blots were hybridized in 1 M NaCl, 10% dextran sulfate, 1% SDS and 0.1 mg/ml salmon sperm DNA with the 635-bp *Eco*RI-*Sac*I fragment of human preprorenin cDNA [15] or with the 420-bp *Alu*I-*Alu*I fragment of mouse β-actin cDNA [16] labeled with (α-^{32}P]dCTP. After hybridization, the membranes were washed sequentially in 2×SSC (1×SSC equals 0.15 M NaCl, 0.015 M sodium citrate) at room temperature for 10 min, 2×SSC, 1% SDS at 60°C for 1 h, and 0.1×SSC at room temperature for 1 h. After autoradiography, relative mRNA levels were determined by densitometric scanning.

3. RESULTS AND DISCUSSION

Expression plasmids coding for human preprorenin and a mutant deleted in its entire N-terminal prosegment were introduced into CHO cells. The cells transfected with the expression plasmid of native preprorenin, pSVDPRnPA33, secreted almost exclusively prorenin (table 1) as previously described [10]. The small amount of active renin in the medium of the transfectant is probably due to the intrinsic activity of prorenin and not due to specific intracellular processing [17,18] because the labeling experiment did not reveal conversion of prorenin to renin (see below). The cells transfected with the mutant plasmid, pSVDΔPRn, secreted active renin, whose activity was about 20-fold lower than that of prorenin after trypsin activation from the pSVDPRnPA33 transfectant (table 1). The renin content in the medium of the pSVDΔPRn transfectant was also about 20-fold lower than that of prorenin in the medium of the pSVDPRnPA33 transfectant (table 1). So these two enzymes had little difference in specific activity after trypsin activation.

Secretion of prorenin and renin from the transfectants was also investigated by radiolabeling with [^{35}S]methionine followed by immunoprecipitation with anti-renin antiserum. The cells transfected with the native preprorenin construct secreted a doublet of 47 kDa and 46 kDa forms of prorenin (fig.2, lane 1; two bands are detectable in a film derived from a shorter exposure, although these are unable to be resolved in this figure). On the other hand, the cells transfected with the mutant construct secreted a doublet of 43 kDa and 42 kDa forms of renin (lane 2). The different forms of prorenin and renin were thought to reflect differences in glycosylation [19]. The densitometric scanning revealed that the secreted amount of renin from the mutant transfectant was also about 20-fold lower than that of prorenin from the native one.

To discover if the difference in secretion observed between those two transfectants was not due to differences in transfection efficiency or in transcription efficiency of the introduced renin cDNA, mRNA levels in the cells were compared by Northern blot analysis (fig.3). The renin mRNA level of the pSVDΔPRn transfectant was about half of the level of the pSVDPRnPA33 transfectant by densitometric scanning. The levels of β-actin mRNA used as control were similar in the two transfectants. Thus, it was revealed that the renin mRNA levels had a much smaller dif-

Table 1

Renin activities and renin contents in the media of transfected CHO cells

Expression plasmid	Renin activity (ng AI/ml per h)	Prorenin activity (ng AI/ml per h)	Total renin concentration (ng/ml)	Specific activity (mg AI/mg per h)
pSVDPRnPA33	41.3 ± 8.06	1750 ± 271	49.5 ± 6.87	36.1 ± 4.39
pSVDΔPRn	95.5 ± 12.0	0	2.97 ± 0.19	32.2 ± 3.56
pSVD	0	0.30 ± 0.25	0	–

Cells were incubated in 60 mm plates and after 48 h, medium was harvested and renin activity and renin content were assayed. Values are the means ± SD of three experiments. pSVD is the expression vector which does not contain preporenin cDNA

[a] Specific activity of total renin

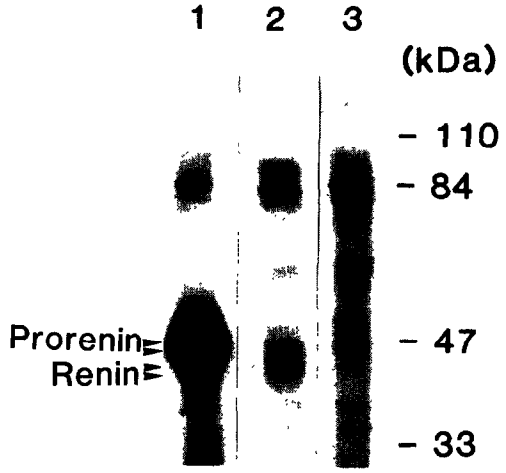

Fig.2. Radiolabeling of secreted prorenin and renin. The cells transfected with pSVDPRnPA33 (lane 1), pSVDΔPRn (lane 2) and pSVD (lane 3) were labeled with [^{35}S]methionine. The culture media were immunoprecipitated with anti-renin antiserum. 0.4 ml and 0.2 ml of media of the pSVDΔPRn transfectant and other transfectants, respectively, were used for immunoprecipitation. In lane 2, a band slightly smaller than that of prorenin is non-specific as it appears in the control cells (lane 3).

ference between these two transfectants than the amounts of renin protein secreted into the medium.

These results suggest that the prosegment plays an important role in the secretory process of renin. One possible function of the prosegment in renin secretion is that it acts as a linker peptide between the signal peptide and the mature renin in order to efficiently cleave the signal peptide. Deletion analyses have recently shown that the 5 amino acid and 6 amino acid N-terminal prosegments of proapolipoprotein AII [4] and proparathyroid hormone [5], respectively, facilitate accurate and efficient signal function. Another possible function of the prosegment is guiding correct folding of the enzyme. Ikemura et al. have shown that the prosegment of prosubtilisin is essential for guiding the enzymatically active conformation of subtilisin [6]. In the case of renin, we have recently shown that some amino acid substitutions in the prosegment reduce the secreted amount of prorenin, probably due to the inefficiency of its folding [20]. However, in the present study, a small but significant amount of fully active renin was secreted by the cells transfected with the deleted construct. This indicates that the prosegment is important but is not necessary for the correct folding of renin.

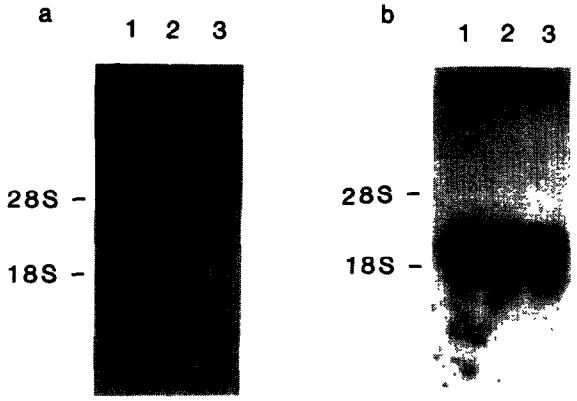

Fig.3. Northern blot analysis using [α-^{32}P]dCTP-labeled human preprorenin cDNA (a) or mouse β-actin cDNA (b) as probe. Total RNA (10 μg) was isolated from CHO cells transfected with pSVDPRnPA33 (lane 1), pSVDΔPRn (lane 2) and pSVD (lane 3) and analyzed by Northern blotting. In (a) the hybridizing RNA in lane 2 is slightly smaller than that in lane 1 due to the deletion of the sequence coding for the prosegment of preprorenin.

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