A 45 000 molecular weight human renin precursor is synthesized in a cell-free translation system

MARC PARMENTIER, TADASHI INAGAMI* AND ROLAND POCHET
Laboratoire d'Histologie, Faculté de Médecine, Université Libre de Bruxelles, Brussels, Belgium, and *Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, Tennessee, U.S.A.

(Received 23 March 1983; accepted 26 May 1983)

Summary
1. Human kidney mRNA species were isolated and fractionated through a continuous sucrose gradient ultracentrifugation.
2. mRNA fractions were translated by using a rabbit reticulocyte lysate and [35S]methionine as tracer. Double immunoprecipitation was carried out with highly specific anti-human renin and anti-rabbit γ-globulin antisera.
3. A 15S mRNA has been found to direct synthesis of a 45 000 molecular weight protein immunoprecipitable with anti-human renin. This protein is considered to be the ultimate precursor of renin (preprorenin).

Key words: enzyme precursor, kidney, messenger RNA, renin, translation in vitro.

Introduction
Renin (EC 3.4.99.19) is a carboxyl-protease, the only known action of which is to catalyse the conversion of angiotensinogen to angiotensin I. Renin has been claimed to exist in several forms. Human active renin extracted from human kidney has been shown to exist with apparent molecular weights of 41 000 [1], 44 000 [2], 50 000 [3] and 52 000 [2]. Inactive forms of renin do exist and constitute 60–90% of the total renin concentration in normal human plasma [4]. The term 'inactive renin' seems to comprise diverse species of related substances with molecular weights ranging from 40 000 to 60 000 [4]. One of these substances seems to be the inactive zymogen of renin which may be derived from the nascent prepro-form. Recently such precursors for renin have been demonstrated by translation in vitro of mRNA isolated from mouse submaxillary gland [5–8] and from mouse kidney [6, 9]. The amino acid sequence deduced from the nucleic acid sequence of the cDNA for the mouse preprorenin has permitted the calculation of the molecular weight of the precursor of mouse renin as 44 209 [8]. In this study we have looked for the molecular weight of human renal renin synthesized in vitro.

Materials and methods
Human renal renin was purified as previously reported [1]. Dutch-belted rabbits were used to produce antibodies to the purified enzyme [10]. Fresh human kidney was obtained from a patient operated on for lithiasis and pyelonephritis. The inferior pole of the kidney, macroscopically unaffected, was frozen in liquid nitrogen after a thorough but rapid rinse in 0.9% NaCl solution. The frozen tissue (6 g) was homogenized in 5 vol. of sodium acetate buffer (10 mmol/l), pH 5.0, containing LiCl (3 mol/l), urea (6 mol/l), 0.5% sodium dodecyl sulphate (SDS), and 200 μg of heparin/ml and stored overnight at 0°C. The frozen tissue (6 g) was homogenized in 5 vol. of sodium acetate buffer (10 mmol/l), pH 5.0, containing LiCl (4 mol/l)/urea (8 mol/l) [11] and incubated for 30 min at 37°C with proteinase K (1 μg/ml). Total RNA was extracted by the phenol/chloroform method [12]. Poly(A)-rich RNA was purified by poly(U) Sepharose chromatography and fractionated through a linear gradient centrifugation [5–30% sucrose in Tris–HCl (10 mmol/l), pH 7.5, NaCl (10 mmol/l) and EDTA (1 mmol/l)] at 60 000 rev./min for 5 h on a SW65 Ti rotor [13]. Twenty fractions of 200 μl were collected under
u.v. monitoring, precipitated, freeze-dried and used for translation in vitro in rabbit reticulocyte lysate. mRNA (100-300 ng) and 30 μCi of [35S]methionine were added to 25 μl of lysate and the mixture was incubated for 90 min at 30°C. Reaction was stopped by adding 400 μl of 1% DOC/1% Triton X-100/0.1% methionine, and hot trichloroacetic acid precipitable radioactivity in 5 μl aliquots was measured. The translation mixture was adjusted to 50 mmol/l Tris-HCl (pH 8.5), 150 mmol/l NaCl, and subjected to immunoprecipitation. The first incubation was performed overnight at 4°C with rabbit anti-human renal renin used at 1:3000 final dilution. Sheep anti-rabbit immunoglobulin was added to this mixture to a 1:300 dilution and the mixture was incubated overnight at 4°C. Immunoprecipitate pellets were recovered with a Beckman Microfuge B, and washed three times in distilled water, dissolved in 70 μl of electrophoresis sample buffer [Tris-HCl (0.1 mol/l), pH 8.0, EDTA (2 mmol/l), 1% SDS, 2% mercaptoethanol, 20% sucrose, 0.1% phenol red] and heated for 2 min at 100°C. Portions (30 μl) were used for a SDS discontinuous polyacrylamide gel electrophoresis on a 12.5% slab gel. Electrophoresis gels were treated with Enlightening solution (NEN) for half an hour, dried and exposed for 6 weeks at -80°C with XAR5 Kodak films. Gels were calibrated by using 14C-labelled proteins of known molecular weight (BRL).

Results
Poly(A)-containing RNA from human kidney were prepared by poly(U) Sepharose chromatography and further size fractionated on sucrose gradient centrifugation. To prevent aggregation, mRNA species were denatured by heating (2 min at 90°C) in low salt medium [Tris-HCl (10 mmol/l), pH 7.5, NaCl (10 mmol/l), EDTA (1 mmol/l)], rapidly cooled and maintained at 2°C during centrifugation [12]. The profile (Fig. 1) showed a classical pattern of undergraded mRNA species with an additional 18S peak corresponding to minor rRNA contamination. Kidney rRNA was fractionated as control in the same run. Aliquots of the fractions collected from the gradient were used for translation in vitro. After electrophoresis of the immunoprecipitable translated material, the radioautography of the gel clearly revealed the presence of an intense major band in several fractions of the gradient (Fig. 2). This band was maximal in fraction 12, corresponding to a 15S mRNA, and had an estimated molecular weight of 45 000. The same band was found in every fraction down to the bottom of the gradient but with decreasing intensity. Some other minor bands could be observed in fractions 11, 12 and 13. They were very weak and detectable only after long exposures. The molecular weights of these bands were evaluated as 56 000, 45 000 and 40 000 respectively.

Discussion
Evidence supporting the existence of different forms of human renin is accumulating [1-4], their molecular weights ranging from 75 000 to 40 000. The present study was intended to determine the molecular weight of the primary product of renin message translation in vitro. Radioautography of electrophoresed 35S-labelled
proteins, after synthesis in vitro and immuno-precipitation by a specific anti-human renin antisem, revealed a major form of renin of molecular weight 45 000. This result is closely related to the molecular weight of mouse submaxillary gland renin precursor (44 209) recently found by complete amino acid sequence [8] and strongly suggests that human kidney renin is produced as a precursor of molecular weight 45 000. Discrepancies between molecular weights deduced from nucleic acids analysis or translation, and those proposed for renin precursors extracted from kidney or plasma, may be explained by the post-synthetic glycosylation of the protease. Renin has indeed been shown to bind concanavalin A [2], and glycosylation is able to increase the size of proteins like cathepsin D by 2000 daltons or more [14]. Other bands though much less intense could also be detected on the gels. The heavier one had an estimated molecular weight of 56 000, corresponding to a protein with 489 amino acids or to 1461 bases for its corresponding mRNA. This size is still compatible with the 15S RNA collected in fraction 12 and human prorenin of such molecular weight (55 000) has already been proposed from pulse labelling studies [15]. However, the small amount detected led us to consider such protein as a contaminant coprecipitated by the antiserum. The radioautography also revealed weak bands of molecular weight 40 000 or less. The hypothetical presence of small amounts of processed or degraded renin in such experiments might result from the action of different proteases present in rabbit reticulocyte lysates [16] and cleaving part of the 45 000 prorenin to forms of lower molecular weight. In conclusion we propose the human kidney renin is synthesized as a precursor of molecular weight 45 000.

Acknowledgements

We thank Professor J. Desclin for helpful discussion. M.P. is Aspirant du Fond National de la Recherche Scientifique of Belgium. This work was partially supported by a grant from Foundation Médicale Reine Elisabeth (Belgium).

References


