

RENIN IS SYNTHESIZED AS A 50 000 DALTON SINGLE-CHAIN POLYPEPTIDE IN CELL-FREE TRANSLATION SYSTEMS

Knud POULSEN, Jens VUUST⁺, Stig LYKKEGAARD, Arne HØJ NIELSEN and Torben LUND⁺

Institute for Biochemistry, The Royal Dental College, Jagtvej 160, DK-2100 Copenhagen Ø and ⁺Department of Molecular Biology, University of Aarhus, DK-8000 Aarhus C, Denmark

Received 20 November 1978

1. Introduction

A number of eukaryotic secretory proteins are synthesized as molecules with an amino-terminal extension of 16–25, mainly hydrophobic, amino acid residues, when their mRNAs are translated in cell-free systems devoid of microsomal membranes (reviewed [1,2]). This short extension is named the 'pre-' or 'signal' peptide and is thought to be essential for the secretion of these proteins [3]. In addition, many secretory proteins, such as some enzymes and peptide hormones, are derived by cleavage of large precursor chains (proenzymes or -hormones). Thus, in these latter cases, the initial translation products are single-chain pre-proenzymes or pre-prohormones [1,2,4,5].

We have isolated total poly(A)-containing mRNA from renin-rich submaxillary glands of mice. These mRNAs were translated, in the presence of [³⁵S]-methionine, in two mRNA-dependent cell-free systems from wheat germ and reticulocyte lysate, respectively. The radioactive translation products were subjected to immunoprecipitation with anti-renin or pure renin-specific Fab fragments, and characterized by SDS-acrylamide gel electrophoresis. Our data indicate that renin is initially synthesized as a precursor ~10 000 daltons larger than the enzymatically active 40 000 dalton renin. In analogy with other enzyme precursors, this may well represent a pre-prorenin.

2. Materials and methods

Animals were male mice of the Danish Serum Institute strain, whose submaxillary glands contain large amounts of renin [6]. Extraction of RNA from these glands was essentially as in [7] except that RNA was precipitated with 2 M LiCl at –20°C for 20 min (a modification kindly suggested to us by Dr R. D. Palmiter). Purification of mRNA was achieved on an oligo(dT)-cellulose column [8].

A wheat germ extract (S-30) was prepared as in [9] except that the preincubation step was omitted. Cell-free incubations in 500 µl contained (final conc.): 400 µl/ml S-30, 20 mM Hepes-KOH (pH 7.4), 80 mM KCl, 2 mM magnesium acetate, 2 mM dithiothreitol, 50 µM spermine, 250 µM spermidine, 0.1 mM of each amino acid except methionine, 50 µCi/ml L-[³⁵S]-methionine (Amersham, 500–1000 Ci/mmol), 1 mM ATP, 0.1 mM GTP, 8 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase, and 20 µg/ml submaxillary gland mRNA. Incubation was for 50 min at 30°C. Immunoprecipitation was with (NH₄)₂SO₄-fractionated rabbit antiserum to mouse submaxillary gland renin (final conc. 20 µg/ml IgG), followed by an equivalent amount of hog antiserum against rabbit IgG light and heavy chains (DAKO). In some experiments, renin antiserum was replaced by renin specific Fab-fragments purified on an affinity column of matrix-bound renin [10]. The precipitate was washed 3 times in 0.05 M phosphate (pH 7.5), reduced, alkylated and electrophoresed on 7.5% SDS-acrylamide gels [11]. Detection of labelled proteins was with fluorography [12], using Kodak X-omat RXR-5 film.

Abbreviations: SDS, sodium dodecylsulphate; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

A rabbit reticulocyte lysate was prepared as in [13] and endogenous mRNA was removed with micrococcal nuclease [14], followed by chromatography on Sephadex G-25 [5]. Incubations (500 μ l) contained: 400 μ l/ml lysate, 16 mM Hepes-KOH (pH 7.4), 30 μ M haemin, 100 mM KCl, 1.4 mM magnesium acetate, 80 μ M spermidine, 6 mM 2-aminopurine, 1 mg/ml calf-liver tRNA and amino acids, ATP, GTP, creatine phosphate and mRNA as in the wheat germ assays. Antirenin (20 μ g/ml) or Fab-fragments (2 μ g/ml) was present during the incubation (60 min at 30°C) [5]. Further analysis was as for the wheat germ samples, except that the immunoprecipitate was washed more thoroughly, as in [15].

3. Results

An SDS-acrylamide gel electrophoretogram of the total submaxillary gland mRNA translation products in a wheat germ assay is shown in fig.1 (lane 1). Most of the synthesized proteins are 40 000 daltons or lower. Addition of antirenin and precipitation of the antigen-antibody complex with anti-IgG, concentrated

a distinct band (fig.1, lane 2) of 50 000 daltons (fig.3); two weaker bands, one with a slightly higher molecular weight and one of ~40 000 daltons, were also seen. If pure 40 000 dalton renin was added in excess (50 μ g/ml) before addition of antirenin, the 50 000 dalton band almost disappeared (fig.1, lane 3) whereas the two weaker bands were still present, indicating that they are not renin, but proteins unspecifically adsorbed to the immune complex. Their unspecific nature could also be directly demonstrated by replacing antirenin with nonimmune IgG (fig.1, lane 4) in which case no band was observed at the 50 000 dalton position, whereas the two weaker bands were unchanged. Pure renin-specific Fab-fragments added instead of the antirenin, revealed the same 50 000 dalton form of renin (fig.1, lane 5) which could be displaced by unlabelled 40 000 dalton renin without affecting the unspecific bands (fig.1, lane 6).

For an independent confirmation of the above results, submaxillary gland mRNA was also translated in a reticulocyte lysate cell-free system. In the total translation products obtained in the presence of antirenin, two bands were seen (fig.2, lane 1), of 50 000 daltons and slightly above 40 000 daltons, respec-

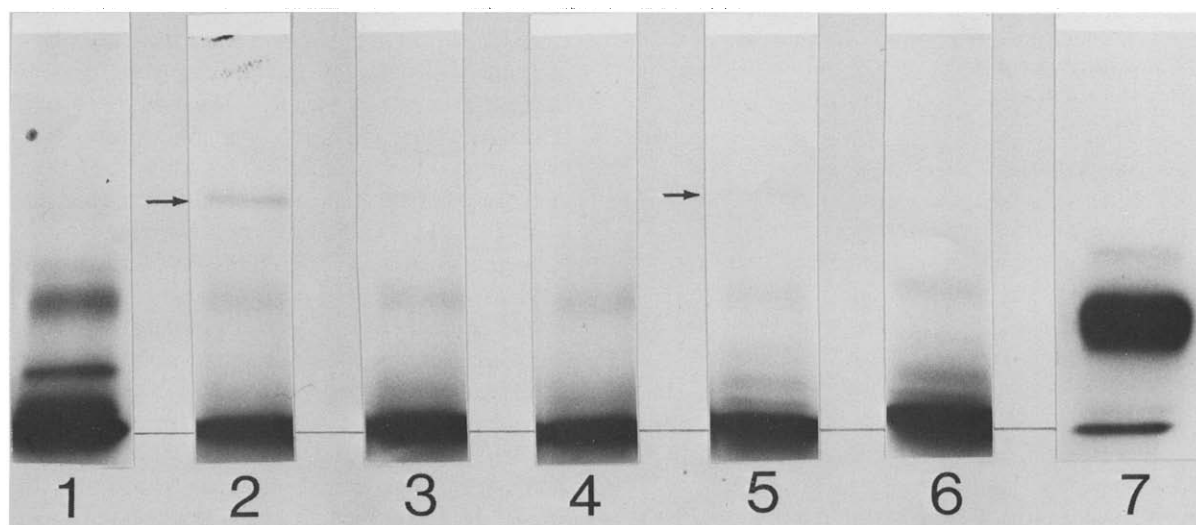


Fig.1. SDS-polyacrylamide gel electrophoresis, followed by fluorography for 105 days, of translation products and their immunoprecipitates obtained in a wheat germ cell-free assay. (1) Total translation products (4 μ l assay mixture). (2) Immunoprecipitation with antirenin of 80 μ l assay mixture. (3) As (2) in the presence of an excess of pure 40 000 dalton renin. (4) Control precipitation of 80 μ l assay mixture with nonimmune IgG. (5) Immunoprecipitation with renin-specific Fab-fragment of 80 μ l assay mixture. (6) As (5) in the presence of pure 40 000 dalton renin. (7) Pure 125 I-labelled 40 000 dalton renin [16]. The arrows indicate the renin precursor.

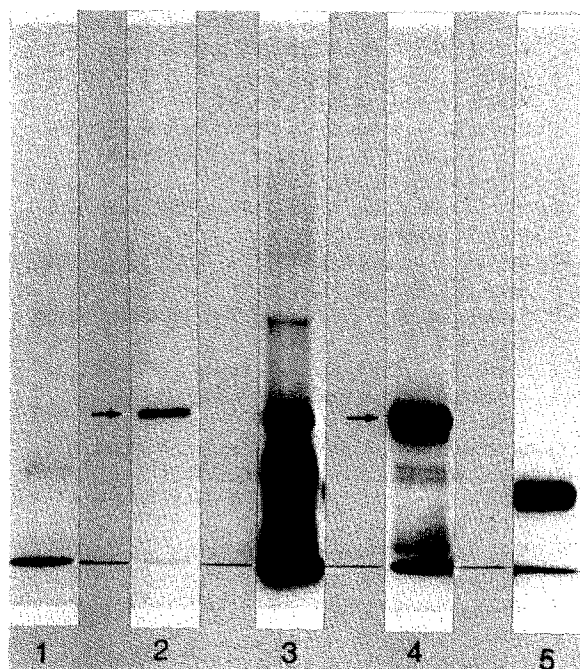


Fig.2. SDS-polyacrylamide gel electrophoresis, followed by fluorography for 17 days, of translation products and their immunoprecipitates obtained in reticulocyte lysate cell-free assays. (1) Total translation products synthesized in the presence of antirenin (12 μ l assay mixture). (2) Immunoprecipitation with antirenin of 250 μ l assay mixture shown in (1). (3) Total translation products synthesized in the presence of Fab-fragments of antirenin (12 μ l assay mixture). (4) Immunoprecipitation with Fab-fragments of 250 μ l assay mixture shown in (3). (5) Pure 125 I-labelled 40 000 dalton renin [16]. The arrows indicate the renin precursor.

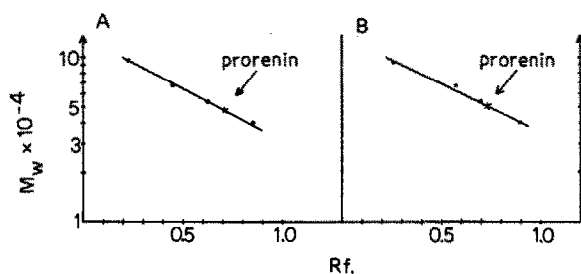


Fig.3. Molecular weight determination by 7.5% SDS-polyacrylamide gel electrophoresis. Molecular weight markers (·) were α 1 (I) from rat-skin collagen, human albumin, heavy chain of IgG and 40 000 dalton renin. The position of the renin precursor is marked (x). (A) Data from reticulocyte lysate experiments. (B) Data from wheat germ extract experiments.

tively. When the antigen-antibody complex was precipitated with anti-IgG, only one distinct band of 50 000 daltons was obtained (fig.2, lane 2; fig.3). An assay carried out in the presence of Fab-fragments of antirenin yielded the same translation products (fig.2, lane 3), but in larger amounts because antirenin inhibited translation to some extent. Following precipitation with anti-IgG, a distinct and very dominant band of 50 000 daltons was obtained (fig.2, lane 4). A few weak bands with lower molecular weights were also seen, probably representing unspecifically-bound material as in the wheat germ experiments.

4. Discussion

The reliability of the immunological identification of the 50 000 dalton translation product as a form of renin was checked in the following ways. First, both renin antiserum and pure antirenin Fab-fragments, whose specificities have been documented [10,16], were used and gave identical results. Second, an excess of pure authentic renin inhibited binding of the 50 000 dalton molecules. Third, it was not bound by nonimmune rabbit IgG. In fact, it was the only labelled translation product that bound specifically to antirenin serum and Fab-fragments. Full reduction and alkylation before gel electrophoresis excludes binding of renin to other proteins. Furthermore, it is unlikely that the 50 000 dalton molecule is derived from an even larger precursor, since the 2 cell-free systems used presumably do not contain the necessary processing enzymes [1,4], except that in some cases the initiating methionine may be cleaved off [4]. Thus, our data strongly indicate that renin, in analogy with other acid proteases (e.g., pepsin and chymosin) [17], is synthesized as a single-chain precursor polypeptide, a prorenin or maybe a preprorenin. In contrast to propepsin (pepsinogen), however, prorenin is converted inside the cell to the 40 000 dalton, enzymatically-active renin [18]. It is released to the blood in the latter form, but appears there also as higher molecular weight moieties [19,20], which probably represent renin bound to some of the plasma protease inhibitors: α_2 -macroglobulin, inter- α -trypsin inhibitor, α_2 -antithrombin and α_1 and β_1 lipoproteins [21].

The binding to the protease inhibitors is probably the first step in the elimination of renin from the organism. The binding to the lipoproteins might also facilitate its transport into the arterial wall and the tissues.

Acknowledgements

We thank Professor B. Foltmann and Professor H. Klenow for valuable discussions in planning the experiments, Professor K. A. Marcker for stimulating discussions of the manuscript, and Dorte Abildsten and Morten Meibom-Larsen for valuable technical assistance. This work was supported by The Danish Heart Association, Danish Foundation for the advancement of Medical Science, P. Carl Petersen's Fond, F. L. Smidth and Co. A/S's Jubilæumsfond, Daell Fonden and the Danish Medical Research Council.

References

- [1] Campbell, P. H. and Blobel, G. (1976) FEBS Lett. 72, 215–226.
- [2] Patzelt, C., Chan, S. J., Duguid, J., Hortin, G., Keim, P., Heinrichson, R. C. and Steiner, D. F. (1977) Proc. 11th FEBS Meet. 47, 69–78.
- [3] Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. 67, 835–851.
- [4] Palmiter, R. D., Thibodeau, S. N., Gagnon, J. and Walsh, K. A. (1977) Proc. 11th FEBS Meet. 47, 89–101.
- [5] Palmiter, R. D., Gagnon, J., Ericsson, L. H. and Walsh, K. A. (1977) J. Biol. Chem. 252, 6386–6393.
- [6] Werle, E., Vogel, R. and Godel, L. T. (1957) Arch. Exp. Pathol. Pharmacol. 230, 236–244.
- [7] Rowe, D. W., Moen, R. C., Davidson, J. M., Byers, P. H., Bornstein, P. and Palmiter, R. D. (1978) Biochemistry 17, 1581–1590.
- [8] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
- [9] Roberts, B. E. and Paterson, B. M. (1973) Proc. Natl. Acad. Sci. USA 70, 2330–2334.
- [10] Lykkegaard, S. (1979) Acta Pathol. Microbiol. Scand. in press.
- [11] Maizel, J. F. (1971) Methods Virol. 5, 179–189.
- [12] Bonner, W. H. and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88.
- [13] Housman, D., Jacobs-Lorena, M., Rajbhandary, U. L. and Lodish, H. E. (1970) Nature 227, 913–918.
- [14] Pelham, H. R. B. and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247–256.
- [15] Jones, R. E. and Grunberger, D. (1978) Arch. Biochem. Biophys. 188, 476–483.
- [16] Malling, C. and Poulsen, K. (1977) Biochim. Biophys. Acta 491, 532–541.
- [17] Foltmann, B. and Pedersen, V. B. (1977) Adv. Exp. Med. Biol. 95, 3–22.
- [18] Nielsen, A. H., Lykkegaard, S. and Poulsen, K. (1979) Biochim. Biophys. Acta, in press.
- [19] Malling, C. and Poulsen, K. (1977) Biochim. Biophys. Acta 491, 542–550.
- [20] Nielsen, A. H., Malling, C. and Poulsen, K. (1978) Biochim. Biophys. Acta 534, 246–257.
- [21] Poulsen, K., Krøll, J., Nielsen, A. H., Jensenius, J. and Malling, C. (1979) Biochim. Biophys. Acta, in press.