Evidence from peptide mapping for a human renin zymogen

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Data obtained from peptide mapping of the active and inactive forms of human renin show that there are extensive regions of common sequence in the two forms of the enzyme, and are consistent with the hypothesis that inactive renin is a renin zymogen.

Peptide mapping *Pro-hormone* *Pro-renin* *Renin*

1. INTRODUCTION

Renin (EC 3.4.99.19) is a highly specific aspartic proteinase which is synthesized mainly in the juxtaglomerular cells of the afferent glomerular arteriole of the kidney. It is released from the kidney into the circulation where it cleaves the α_2-globulin angiotensinogen to produce the inactive decapeptide angiotensin I. Subsequently, angiotensin I is cleaved by a peptidyl dipeptide hydrolase (converting enzyme) to give the vasoactive octapeptide, angiotensin II, which has numerous actions concerned with sodium and fluid homeostasis and with the maintenance of blood pressure [1,2].

Many proteins are synthesized as inactive precursors or zymogens which are subsequently converted to their active forms by limited proteolysis. This is true for a large number of extracellular proteinases including pepsin and chymosin, the best characterised of the aspartic proteinases [3]. Recently, it has become apparent that renin is also synthesized as a precursor [4–8].

In man, an inactive form of renin is present both within the kidney and in the circulation [9–12]. Although this form of the enzyme has been called prorenin, little evidence has been presented to show that it is actually a biosynthetic precursor of renin. In extracts of some animal kidneys, renin is associated with a 'renin-binding protein' [13–15] and thus the possibility that human inactive renin is a complex of active renin with a renin inhibitor could not be excluded.

We have recently purified microgram quantities of inactive renin from human renal cortex and shown that it consists of a single polypeptide chain [16]. As expected for a renin zymogen, human inactive renin (M_r 48000) is slightly larger than the active form of the enzyme (M_r 40000) and can be activated by limited proteolysis. We report direct evidence obtained from peptide mapping that the active and inactive forms of renin are structurally related.

2. MATERIALS AND METHODS

2.1. *Reagents*

Chemicals and other materials were obtained either from the sources described in the appropriate references or from BDH Chemicals, Poole, Dorset, except for the following: bovine serum albumin (BSA), aldolase, pyruvate kinase and carbonic anhydrase were from Boehringer (London), Lewes, Sussex; ovalbumin, β-lactoglobulin, and Bolton–Hunter reagent (*N*-succinimidyl-3-(4-hydroxyphenyl)propionate) were from Sigma, Poole; V8 proteinase from *Staphylococcus aureus* was from Miles Laboratories, Stoke Poges, Slough; labelled Bolton–Hunter reagent (*N*-succinimidyl-3-(4-hy-

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droxy-5-[^{125}I]iodophenyl)propionate; 2000 Ci/mmol) was from Amersham International, Bucks.

Active and inactive renin were purified from human renal cortex as described previously [16,17].

2.2. *Polyacrylamide gel electrophoresis (PAGE)*

Electrophoresis in the presence of 0.1% (w/v) sodium dodecyl sulphate (SDS) was carried out using the method of [18].

2.3. *Radiolabelling of proteins*

Samples (2 μg) of active renin, inactive renin, and a mixture of standard proteins (0.4 μg each of pyruvate kinase, ovalbumin, aldolase, carbonic anhydrase and β-lactoglobulin), dissolved in 100 μl of 80 mM triethanolamine/HCl (pH 8.5)/2% (w/v) SDS, were heated at 100°C in a boiling water bath for 3 min and then cooled to 4°C. 1 μg of each protein sample (50 μl) was reacted with 300 pmol of labelled Bolton–Hunter reagent. In each case, the reagent (600 μCi), dissolved in 50 μl of benzene/0.2% (v/v) dimethylformamide, was pipetted into a small glass vial (5 × 15 mm) and then dried by evaporation of the solvent at room temperature under a flow of nitrogen. The sample was added to the dried reagent and the reaction mixture shaken for 30 min at 4°C. The reaction mixture was then transferred to a second glass vial containing 10 μmol of unlabelled Bolton–Hunter reagent and shaken for a further 30 min at 4°C. In order to prevent the subsequent labelling of carrier proteins, unchanged Bolton–Hunter reagent was reacted with 50 μl of 100 mM Tris/HCl (pH 8.5) for 10 min at 4°C. Finally, 100 μl of 100 mM Tris/HCl (pH 8.5), containing 200 μg BSA as carrier protein, was added to the mixture.

2.4. *Purification of labelled proteins*

The labelled proteins were separated from the other labelled products of the acylation reaction (acylated Tris and 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionic acid) by precipitation with 1 ml of ice-cold 10% (w/v) trichloroacetic acid. In each case, the precipitated protein was allowed to stand for 15 min on ice and was then collected by centrifugation at 1000 × g and 4°C for 5 min. Each precipitate was washed with 1 ml of ice-cold 10% (w/v) trichloroacetic acid and then with 2 × 1 ml of ice-cold acetone.

The labelled proteins were further purified by PAGE in the presence of 0.1% (w/v) SDS. Each protein precipitate was dissolved in 50 μl of sample buffer (72.5 mM Tris/HCl (pH 6.8)/3% (w/v) SDS/10% (v/v) glycerol/5% (v/v) 2-mercaptoethanol/0.002% (w/v) bromophenol blue), and electrophoresis carried out using polyacrylamide rod gels (5 mm diameter; 3% stacking gel, 10% resolving gel). After electrophoresis, the gels were frozen using powdered dry ice. Each gel was sliced into 1 mm segments and the segments counted for ^{125}I.

Labelled proteins were eluted from the polyacrylamide gel by crushing the appropriate gel segments with a glass rod and incubating the gel fragments for 24 h at 4°C with 300 μl of 100 mM ammonium bicarbonate/0.01% (w/v) BSA (elution buffer). In each case, the gel slurry was then poured into a 1 ml disposable pipette tip containing glass wool and the liquid separated from the gel fragments by centrifugation for 2 min at 500 rpm in a benchtop centrifuge. The gel slurry was rinsed with a further 200 μl of elution buffer and centrifuged again for 2 min at 500 rpm. The combined eluates containing the labelled proteins were then frozen and stored at −20°C.

2.5. *Peptide mapping by analysis of V8 proteinase digests using high-performance liquid chromatography (HPLC)*

HPLC solvents were argon degassed for each experiment. Trifluoroacetic acid (0.1%, w/v) was filtered through a 0.2 μm Millipore filter.

Samples (200000 cpm) of labelled active and inactive renin in 200 μl of 100 mM ammonium bicarbonate/0.01% (w/v) BSA were exhaustively digested by incubation with 5 μl of V8 proteinase solution (1 mg/ml in 100 mM ammonium bicarbonate) for 24 h at 37°C and then with a further 5 μl of the proteinase solution for 6 h at 37°C.

Analysis of the protein digests was carried out using a Gilson model 303 computer controlled gradient pumping HPLC system (Scotlab, Bellshill, Scotland) fitted with a μBondapak C_{18} reverse-phase column (Waters, Hartford). Each protein digest was lyophilised and resuspended in 25 μl 0.1% (w/v) trifluoroacetic acid. 20 μl of this solution was applied to the column using a valve loop injector and the chromatogram developed over 80 min using a 0–40% gradient of isopropanol in

0.1% (w/v) trifluoroacetic acid at a flow rate of 1 ml/min. 300-μl fractions were collected and counted for ^{125}I.

3. RESULTS

Standard microscale peptide mapping techniques involving the detection of resolved peptides using ninhydrin or fluorescamine generally require milligram quantities of protein as starting material [19]. Since only microgram quantities of the active and inactive forms of renin were available for comparison by peptide mapping, it was necessary to label the proteins using radioiodinated Bolton–Hunter reagent. This reagent labels both lysine and terminal amino groups [20].

The labelled proteins were separated from other labelled products of the reaction by precipitation with trichloroacetic acid followed by PAGE in the presence of 0.1% (w/v) SDS (fig.1). The active renin preparation contained both labelled renin (Peak I; M_r 41000) and a smaller component (Peak II; M_r 17000) which we previously observed in preparations of renin [17]. The inactive renin preparation contained a single labelled polypeptide of M_r 49000. The labelled active and inactive renin were recovered from the gels in yields of 63 and 73%, respectively.

A structural comparison of the two forms of renin was carried out by digestion of the labelled proteins with V8 proteinase followed by analysis of the digests by reverse-phase HPLC. Nine labelled peptides were resolved by HPLC 'mapping' of active renin (fig.2). Each of these peptides was also present in the digest of inactive renin, indicating that there are extensive regions of common sequence in the two forms of the enzyme. The digest of inactive renin also contained three labelled peptides (III, IV and XI) which were not present in the digest of active renin. These data are clearly consistent with the hypothesis that inactive renin is a true renin zymogen.

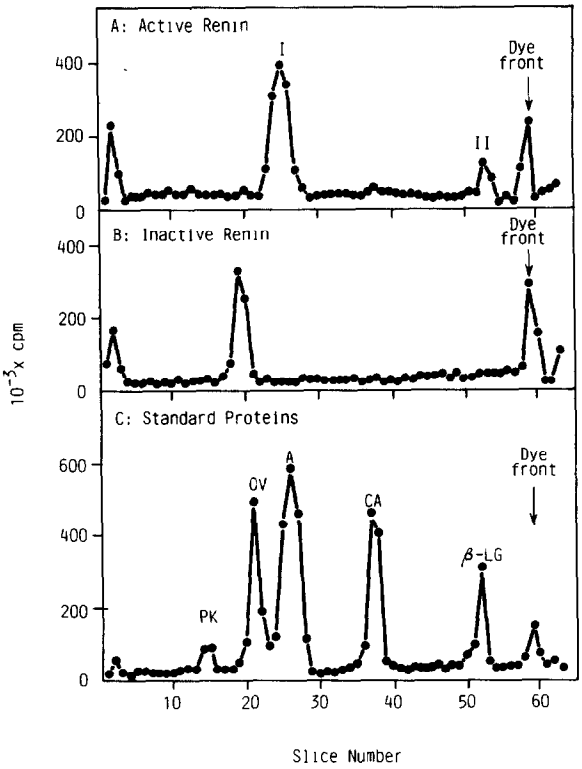

Fig.1. SDS–PAGE of radiolabelled proteins. Discontinuous electrophoresis was carried out in rod gels (stacking gel, 3% polyacrylamide; resolving gel, 10% polyacrylamide). After electrophoresis was complete, the gels were sliced into 1-mm segments and the segments then counted for ^{125}I. A, labelled active renin preparation; B, labelled inactive renin preparation; C, labelled standard M_r proteins: PK, pyruvate kinase (57000); OV, ovalbumin (43000); A, aldolase (40000); CA, carbonic anhydrase (29000); β-LG, β-lactoglobulin (17500).

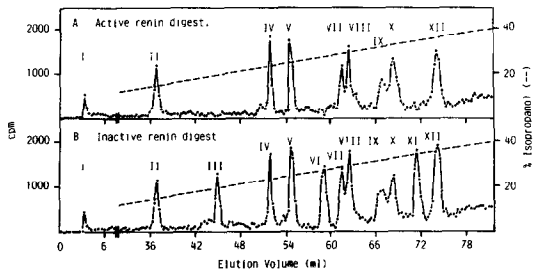

Fig.2. HPLC analysis of V8 proteinase digests of the active and inactive forms of renin. V8 proteinase digests of ^{125}I-labelled active and inactive renin (approx. 170000 cpm of each preparation) were analysed by HPLC. Each digest, dissolved in 20 μl of 0.1% (w/v) trifluoroacetic acid, was applied to a μBondapak C_{18} reverse-phase column and the chromatogram developed over 80 min using a 0–40% gradient of isopropanol in 0.1% (w/v) trifluoroacetic acid at a flow rate of 1 ml/min. 300-μl fractions were collected and counted for ^{125}I.

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