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PURIFICATION OF HUMAN RENIN

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SUMMARY: Human renin was purified 2,800-fold from a partially purified preparation to an electrophoretically homogeneous state by a series of three different types of affinity chromatography and two additional conventional chromatographic steps at a yield of 9.7%. This amounts to a 420,000-fold purification from a crude kidney extract. This pure human renin preparation has a specific activity of 830 Goldblatt unit/mg and is stable at pH 6.2 and 4°C at least for 3 weeks.

Recognition of the importance of renin (E. C. 3·4·99·19) in blood pressure regulation has led to many attempts for its purification. Very low concentration of this enzyme in the kidney and extreme instability in its partially purified states presented great obstacles to such attempts (1-7). Particularly, the low concentration of renin in the human kidney (8) made its purification very difficult. Since human renin seems to differ from renin of other mammalian species (9), its purification was of considerable importance. Techniques which were successfully applied for the purification of renin from animals (10,11) were not satisfactory to cope with difficulties involved in human renin purification. In order to remove proteases present at a very high concentration in the human kidney, we have devised a purification method which involves a series of affinity and conventional chromatographic steps. An electrophoretically homogeneous human renin was obtained from the partially purified preparation of Haas, Goldblatt and Gipson (2).

MATERIALS AND METHODS

Affinity columns: A hemoglobin-Sepharose column was prepared by the method

Abbreviations: DFP, diisopropylphosphorofluoridate; GU, Goldblatt Unit.

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of Chou et al., (12) by coupling 0.83 g of crystalline bovine hemoglobin to 50 ml (wet volume) of cyanogen bromide activated Sepharose 6B (Pharmacia) gel. Pepstatin-aminohexyl-Sepharose gel was prepared by the method previously published from this laboratory (10). The peptide Gly-His-Pro-Phe-His-Leu-Phe-Val-Tyr was also used as an affinity ligand. It was synthesized by the solid phase method of Manning, Coy and Sawyer (13), deprotected and released from resin by hydrogen fluoride, prepurified on a Sephadex G-25 (Pharmacia) column in water-dioxane-acetic acid (75 : 20 : 5%, v/v) and further purified by partition chromatography on a Sephadex G-25 column equilibrated with the lower layer of butanol-acetic acid-water (5 : 4 : 1, v/v) and eluted with the upper layer of the mixture. This peptide (15 mg) was coupled to 2 ml of cyanogen bromide activated Sepharose 4B (Pharmacia) gel.

Purification: The partially purified human renin preparation, step 7 of Haas, Goldblatt and Gipson (2) with a specific activity of 0.30 GU/mg protein was used as the starting material. The freeze dried material (1930 mg, 580 GU) was dissolved in 0.02 M Na-acetate buffer, pH 6.0 containing 1 M NaCl and the protease inhibitors, 0.1 mM DFP, 5 mM benzamidine, 5 mM Na-tetrathionate and 5 mM EDTA. This solution was applied to the hemoglobin-Sepharose column (2.6 x 22 cm) and was eluted with 1.54 l of 0.02 M Na-acetate buffer, pH 6.0, containing 1 M NaCl and 0.05 mM DFP, then with 0.1 M Tris-acetate buffer, pH 8.6, containing 1 M NaCl and 0.05 mM DFP. Fractions containing renin activity (bracket, Fig. 1) partially segregated from the early protein peak were pooled and applied to the pepstatin-aminohexyl-Sepharose column (2.2 x 16 cm) previously equilibrated with 0.02 M Na-acetate buffer, pH 6.0, containing 1 M NaCl and 0.05 mM DFP. The column was washed with 660 ml of the same buffer, 660 ml of 0.1 M Na-acetate buffer, pH 5.2, containing 1 M NaCl and 0.05 mM DFP (arrow I, Fig. 2) then eluted with a pH gradient generated by allowing 0.1 M acetic acid containing 0.05 mM DFP to mix with 120 ml of 0.1 M Na-acetate buffer, pH 5.2, containing 0.05 mM DFP (arrow II). Additional protease activity and very small amount of renin activity were eluted by 0.5 M Tris-acetate buffer, pH 7.5 (arrow III). The renin containing fractions (bracket, Fig. 2) were immediately adjusted to neutral pH with 2 M Tris-acetate buffer, pH 8.0, pooled, concentrated by pressure filtration using an Amicon filter PM-10, equilibrated with 0.01 M Na-pyrophosphate buffer, pH 6.5, containing 0.1 M NaCl and chromatographed on a Sephadex G-100 column (1.5 x 97 cm) (Fig. 3). Active fractions indicated by the bracket were dialyzed against 0.01 M Na-acetate buffer, pH 5.5, and applied to the octapeptide-Sepharose column (0.9 x 2.9 cm). After a wash with 42 ml of 0.01 M acetate buffer, pH 5.5, an upward pH gradient was applied (at arrow, Fig. 4) by allowing 20 ml of 0.2 M Tris-acetate buffer, pH 7.5, to mix with 20 ml of the initial buffer. Active fractions which emerged early were pooled, dialyzed against 0.02 M Na-phosphate buffer, pH 6.2, and chromatographed on a DEAE-cellulose column (Whatman DE 52, 0.5 x 7.5 cm) first with 32 ml of the same buffer followed (at arrow, Fig. 5) by a linear NaCl gradient generated between 30 ml each of the initial buffer and the same buffer containing 0.2 M NaCl. The renin activity emerged as a symmetrical peak superimposable with a symmetrical protein concentration peak (Fig. 5). Fractions under this peak were used as pure renin.

Assay: Renin activity was determined by the rate of generation of angiotensin I with renin substrate which had been obtained from the plasma of nephrectomized hogs and partially purified (14). After incubation at 37° and pH 6.0, in 0.20 M maleate buffer, containing 7 mM EDTA and 2 mM phenylmethylsulfonylfluoride, angiotensin was determined by the radioimmunoassay of Haber et al. (15). The concentration of renin in terms of the international (Goldblatt) unit was determined by comparison with the "International Reference Preparation of Human Renin" coded 68/356 from the National Institute of Biological Standards and Control, Holly Hill, London (19). Protein con-

TABLE I. Purification of Renin from Human Kidney

Purification Step	Total Protein mg	Specific Activity GU/mg protein	Purification	Yield %
Haas, Step 7	1930	0.30	1 (150)*	100
Hemoglobin-Sepharose	140	3.6	12 (1,800)	88
Pepstatin-Sepharose	4.2	76	250 (38,000)	55
Sephadex G-100	0.50	280	930(140,000)	24
Octapeptide-Sepharose	0.11	550	1800(270,000)	10
DEAE-Cellulose	0.066	830	2800(420,000)	9.7

*Values in parentheses are based on the initial kidney extract of Haas et al. (Ref. 2).

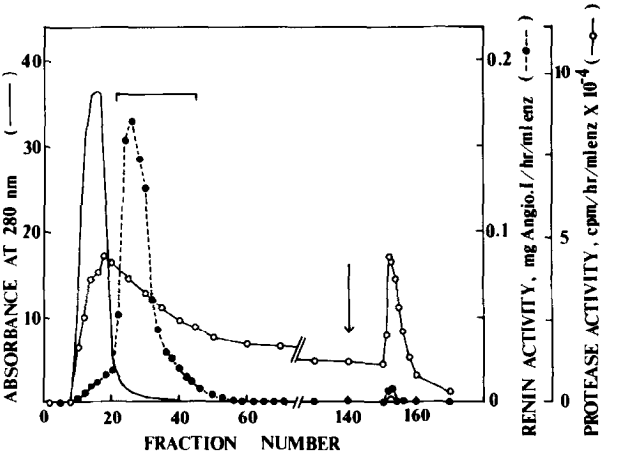

Fig. 1. Affinity chromatography on a hemoglobin-Sepharose column. The elution buffer was changed at the arrow.

centration was determined spectrophotometrically by the method of Waddell (16). Absorbance at 280 nm was also used to follow changes in protein concentration in chromatographic effluent. Protease activity was assayed at pH 5.4 according to the method of Williams and Lin (17) using hemoglobin coupled with [^{14}C]-glycine methyl ester as substrate.

RESULTS AND DISCUSSION

The purification of human renin was accomplished by the 5 step chromatographic procedure summarized in Table I. Starting with 1.93 g of partially

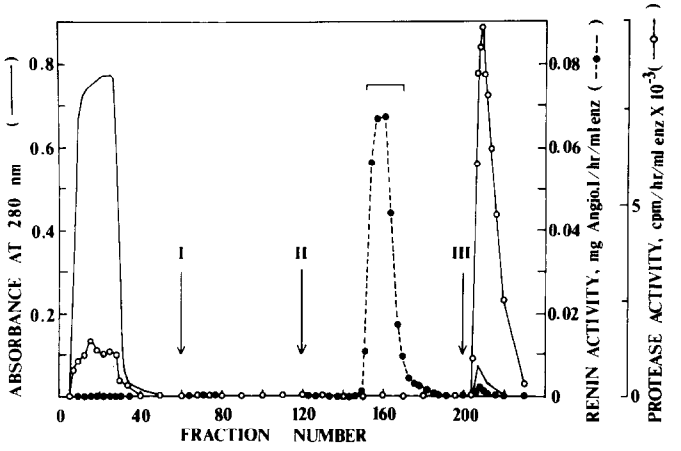

Fig. 2. Affinity chromatography on a pepstatin-Sepharose column.

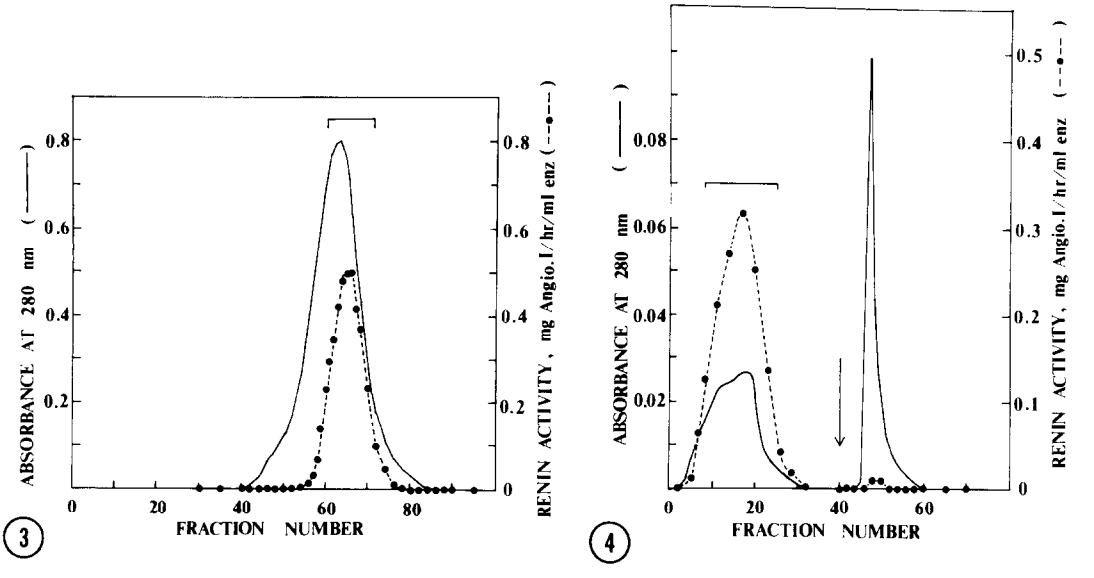

Fig. 3. Gel filtration on a Sephadex G-100 column.

Fig. 4. Affinity chromatography on an octapeptide-Sepharose column.

purified lyophilized material, which was derived from 9 kg of human kidney by the method of Haas, Goldblatt and Gipson (2) approximately 66 μg of renin was obtained with a specific activity of 830 GU/mg protein and representing a 2,800-fold purification. Since the starting material had already been

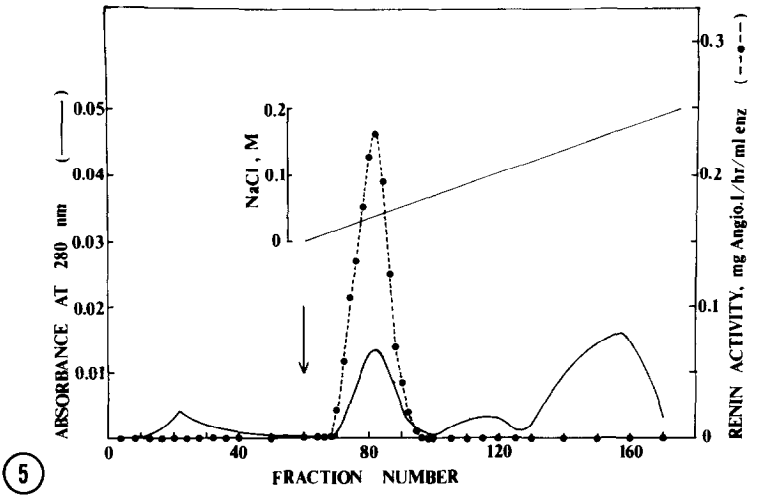

Fig. 5. Chromatography on a DEAE-cellulose column.

Fig. 6. Polyacrylamide gel electrophoresis of human renal renin obtained from DEAE cellulose chromatography. Electrophoresis was performed on 7.5% cross-linked gel at pH 9.5.

purified 150-fold, an overall 420,000-fold purification was obtained compared to the renin (specific activity : 0.002 GU/mg protein) in the first crude extract from human kidneys. The symmetric elution pattern completely superimposable with the protein concentration peak and the single band obtained upon disc electrophoresis (Fig. 6) indicate that this material is a pure human renin preparation. This preparation at a concentration of 10 μg/ml was stable over a period of 3 weeks at pH 6.2 and 4°C.

Our previous method of human renin purification (7) has been modified by employing two additional affinity columns, in order to achieve the complete removal of proteases, which, previously had been the cause of the rapid proteolytic inactivation of human renin. The hemoglobin-Sepharose column originally devised by Chou, Shaper and Gregerman (12) for the separation of

human pseudorenin and renin turned out to be a very useful tool for an efficient removal of proteases. Although this column alone did not remove proteases completely, combination with the pepstatin-aminohexyl-Sepharose column was very effective in producing a protease-free and stable preparation. The complete removal of the protease is the major factor which permitted the completion of the purification of human renin at a relatively high yield. The new affinity column with the synthetic octapeptide was prepared in analogy with those of Poulsen, Burton and Haber (18). This had a very weak affinity to human renin but was effective in removing additional non-renin substance.

The final product was obtained as a single peak in contrast to multiple components obtained in our previous studies (7). Presumably, this is due to the efficient elimination of proteases at the very beginning of the present procedure which may have prevented the formation of partially degraded forms of renin. The present preparation has a specific activity approximately 4 times as high as the previously reported value for the partially purified human renin (7). It did not have detectable protease activity.

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