AN AFFINITY COLUMN FOR RENIN*

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SUMMARY: An affinity column for renin was prepared making use of the strong affinity of pepstatin for renin. Pepstatin is an N-acylated pentapeptide from Actinomycetes with the following structure: isovaleryl-L-valyl-L-valyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid. This peptide was coupled to aminoethylated polyacrylamide gel either directly with the water-soluble carbodiimide, 1-ethyl-3-(dimethylaminopropyl) carbodiimide, or through the N-hydroxy succinimide ester. Submaxillary renin was selectively retained by a small column of the gel and was eluted by a salt gradient to produce a highly pure material. This column was also effective for the purification of renal renin.

Numerous attempts to purify renin in the past have encountered great difficulties due to both the instability of renin under certain conditions and to the extremely small amount of renin present in the kidney (1-5). Affinity chromatography (6) seems to be particularly suited for application to this problem since a specific substance can be retained on and released from an affinity column in a selective manner.

Previously, we have purified renin from submaxillary glands of mice by a conventional column chromatographic method (7) and have obtained the enzyme

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in crystalline form. Using this pure enzyme we initiated a search for competitive inhibitors with high affinity for renin to be used as the ligand of a renin-specific affinity column.

Among the various peptides examined, the pentapeptide pepsin inhibitor pepstatin, isolated from Actinomycetes by Umezana et al. (8), was found to be the most potent. Pepstatin used at 2 μM gave a 50% inhibition of salivary renin activity, as measured by the octapeptide fluorogenic substrate of Reinharz and Roth, at a concentration of 56 μM, pH 5.6 and 37°. Since this substrate has a K_m of 23 μM under these conditions, the K_i of pepstatin should be in the micromolar range. Also, Gross et al., have reported that pepstatin has a potent inhibitory activity to renal renin (9) at a micromolar concentration. These findings strongly indicated pepstatin would be useful as the ligand of an affinity column for the isolation of renin. In the following we report the preparation of such a column and its application to the purification of submaxillary renin.

Pepstatin, an N-acylated pentapeptide: isovaleryl-L-valyl-L-valyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid (8), was coupled to aminoethylated polyacrylamide gel (Aminoethyl Biogel-P-150 from BioRad) through the activation of the carboxyl group of pepstatin using a water soluble carbodiamide, 1-ethyl-3-(N-dimethylaminopropyl)-carbodiimide (Pierce Chemical Co.). One mmole of pepstatin was dissolved in 200 ml of 5 mM NaOH. Twenty ml of wet Aminoethyl-Biogel-P-150 was then added, followed by 10 mmoles of the water soluble carbodiimide. The mixture was stirred by a gentle magnetic stirring throughout the reaction period at room temperature. The pH of the mixture was maintained at 6.2 by a Radiometer pH-stat. After 2 hours of initial reaction, a second addition of the carbodiimide (10 mmoles) was made and the reaction was

continued for an additional one hour period. The reaction mixture was then allowed to stand overnight without the magnetic stirring. At the end of this period unreacted amino groups on the gel were blocked by a treatment with 10 mmoles of acetic acid and the carbodiimide at pH 7.4 for 2 hours. The gel was washed by several decantations with 0.1 M sodium bicarbonate and water to remove reagents and fines of the gel formed during the reaction. The gel was washed more thoroughly after being packed in a glass column.

Pepstatin-bound polyacrylamide gel was also prepared by reacting the N-hydroxysuccinimide ester of pepstatin with the gel according to a modified technique of Cuatrecasas (6). Two mmoles of pepstatin were dissolved in 80 ml of redistilled dimethyl formamide, 2.4 mmoles of recrystallized N-hydroxysuccinimide (Pierce Chemical Co.) and 2.2 mmoles of dicyclohexylcarbodiimide (Pierce Chemical Co.) were added. The mixture was allowed to react for 2 hours and filtered. The filtrate was added to a suspension containing 100 ml of wet Aminoethyl-Biogel-P-150 gel and 800 ml of 0.1 M phosphate buffer, pH 7.4. After 16 hours of reaction unreacted amino groups on the gel were blocked and the gel was washed as described above.

The amount of pepstatin incorporated by both procedures was determined by quantitating the alanine and valine of the coupled pepstatin by amino acid analysis. Approximately 0.4 - 0.7 μ moles was bound to mg of dry gel corresponding to a recovery of 15 - 17% of pepstatin using the water-soluble carbodiimide. The coupling through the N-hydroxysuccinimide ester of pepstatin gave a slightly better yield of approximately 20%. A column (1.0 x 7.0 cm), of the gel was prepared and thoroughly washed and equilibrated with a 0.02 M Tris-Cl buffer, pH 7.5.

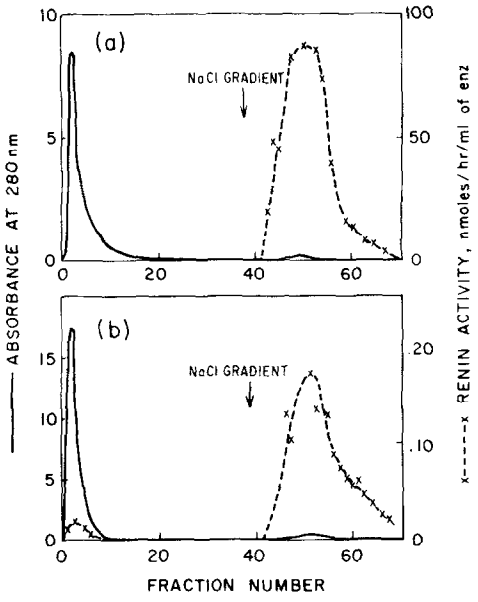

Fig. 1 (a) Affinity chromatography of partially purified submaxillary renin on a column (1.0 x 7.0 cm) of pepstatin-aminoethyl polyacrylamide gel prepared through the N-hydroxysuccinimide ester of pepstatin. The renin was eluted by a salt gradient prepared by allowing 0.02 M Tris-Cl buffer containing 0.1 M NaCl to flow into a 250 ml constant volume mixing chamber filled with the sodium chloride free buffer. The other details are in the text.
(b) Partially purified hog renin was treated in a similar manner.

A crude renin preparation was made from a water extract of submaxillary glands of male mice by streptomycin treatment, ammonium sulfate precipitation and gel chromatography on a column of Sephadex G-100, by a published method (7). Renin fractions (200 mg in protein) from the Sephadex column in the 0.02 M Tris-Cl buffer, pH 7.5, were applied to the affinity column. The column was thoroughly washed with the same buffer until no further protein was eluted, then a shallow gradient of NaCl was applied in the same buffer. Renin activity was readily eluted by the gradient as a very small protein peak as shown in Fig. 1a. The pooled active fraction from the affinity column had a purity higher than 85% as compared with the specific activity of the purest preparation obtained previously. The active fraction

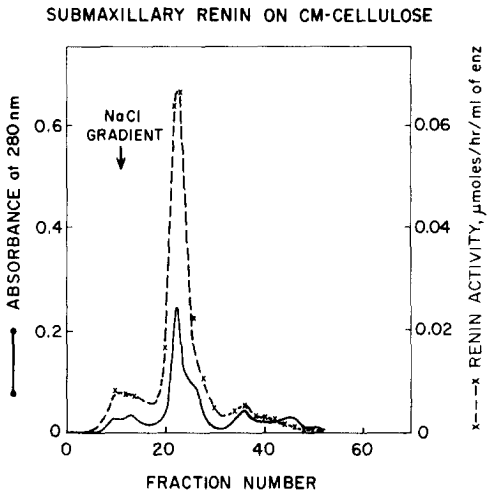

Fig. 2 Ion exchange chromatography of the submaxillary renin fraction from the affinity column (Fig. 1a) on a CM-cellulose column (1.5 x 24 cm). The salt gradient was prepared by allowing 0.02 M acetate buffer, pH 5.4 containing 0.15 M NaCl to flow into a 125 ml constant volume mixing chamber filled with the sodium chloride free buffer.

(12 mg in protein) can be further separated into a single major component and several minor components by a chromatography on a carboxymethyl cellulose column at pH 5.4 eluted with a 0.05 M acetate buffer with NaCl concentration gradient as shown in Fig. 2. Examination of the major component by polyacrylamide gel electrophoresis at pH 9.5 revealed that the component corresponded to renin A separated previously by 5 steps of conventional column chromatography (7).

Thus it is possible to purify a family of the isoenzymes of mouse submaxillary renin by a very simple operation using a pepstatin bound affinity column.

Since pepstatin has a strong affinity to renal renin (9), a preliminary attempt was made to apply this method for the purification of hog renal renin. A crude preparation (100 mg) obtained from Pentex and fractionated with ammonium sulfate (0 - 50% saturation) was treated in a manner similar to

that described above and a renin containing peak was separated from the bulk of non-renin proteins with an approximate 25-fold purification as shown in Fig. 1b. Thus this method may have an important potential use in the purification of renal renin.

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