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SEPARATION OF HUMAN RENAL RENIN AND PSEUDORENIN BY AFFINITY CHROMATOGRAPHY ON HEMOGLOBIN-SEPHAROSE-2B

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Summary

Human renal renin (EC 3.4.99.19) and pseudorenin were easily separated in a single step by affinity chromatography on hemoglobin-Sepharose-2B. Renin and pseudorenin were monitored by their actions on crude and partially purified hog protein renin substrates at neutral and acidic pH and on synthetic labelled polymeric renin substrate. Under the conditions employed (0.1 M sodium acetate (pH 3.5)/1 M sodium chloride at 4°C) renin does not bind to the affinity adsorbent while pseudorenin is effectively bound and can be eluted only after raising the pH to 6.5. Pseudorenin-free renin prepared by this method is devoid of proteolytic activity toward hemoglobin. The chromatographic behavior of renal pseudorenin on hemoglobin-Sepharose-2B is similar to that of cathepsin D.

Introduction

Crude preparations of renin (EC 3.4.99.19) contain a renin-like activity which has been termed pseudorenin. Pseudorenin resembles renin in its ability to form angiotensin I from the synthetic tetradecapeptide renin substrate or from purified hog protein substrate, but does not produce angiotensin from crude protein substrate. In contrast to renin, which is primarily or exclusively produced in the kidney, pseudorenin has been found in 13 different tissues as well as in plasma [1]. Although human renin and hog renin have been isolated free from contamination by pseudorenin by several groups using multiple step ion-exchange and gel filtration procedures [2—4], we are not aware of any

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report of the separation of these enzymes by a simple procedure.

A particular type of affinity column chromatography has been shown to be useful for the rapid and simple purification of wheat proteases [5] and intracellular cathepsin D [6]. The essential step in this purification is the adsorption of the proteases on a Sepharose gel to which a classic protein substrate for proteases, hemoglobin, has been convalently bound. We wish to report here the successful application of this technique to the purification of renin.

Materials and Methods

Bovine hemoglobin was obtained from Pentex (Kankakee, Ill.). Sepharose-2B was from Pharmacia. Human renin (specific activity, 0.13 Goldblatt Unit/mg) was the generous gift of Dr. Erwin Haas and had been prepared by his procedure A [7]. Hog renin (10 units/mg) was from Nutritional Biochemicals.

Hemoglobin-Sepharose-2B adsorbent

The material was prepared by the procedure of Smith and Turk [6]. Hemoglobin was coupled at pH 6.5 for 24 h at 4°C to CNBr-activated Sepharose-2B. The affinity adsorbent contained 0.5—1.5 mg hemoglobin per ml packed wet resin (determined by amino acid analysis after hydrolysis in vacuo in 6 M HCl for 24 h at 110°C).

Crude and purified hog protein renin substrate

Crude hog protein renin substrate was prepared by $(NH_4)_2SO_4$ fractionation of plasma as described by Gould et al. [8]. Partially purified substrate was prepared by dialysis of crude substrate overnight against 0.02 M sodium phosphate (pH 7.5, 4°C), followed by chromatography on DEAE-Sephadex-A-50 (2.5 X 100 cm column). Using a salt gradient (0.02 M sodium phosphate/0—0.5 M NaCl) elution of the hog renin substrate began at 0.15 M NaCl. Localization was by incubation of the fractions with excess hog renin and radioimmunoassay of the angiotensin I formed after incubation.

Affinity chromatography on hemoglobin-Sepharose-2B adsorbent

10 mg human renin (1 unit) was dissolved in 1 ml buffer (0.1 M sodium acetate (pH 3.5)/1 M NaCl) and applied to a hemoglobin-Sepharose-2B column (1 × 10 cm) equilibrated with the same buffer. The column was eluted with buffer (10 ml/h, 2.5 ml fractions) until the eluate had a constant absorbance at 280 nm of less than 0.05. The column was then eluted with 0.1 M Tris · HCl buffer (pH 6.5)/1 M NaCl and further fractions collected (Fig. 1).

Radioimmunoassay for angiotensin I

This assay was performed as described by Haber et al. [9] using crude or partially purified hog renin substrate prepared as described above.

Proteolysis assay

The preparation of the [^{14}C]glycinated hemoglobin substrate and conditions of the assay were as described by Williams and Lin [10].

Protein determinations

These were made by the method of Lowry et al. [11] using bovine serum albumin as the standard.

Results

Although human renin attacks hog renin substrate in crude plasma, pseudorenin does not, since plasma contains a potent pseudorenin inhibitor [1]. Accordingly, the use of purified substrate allowed localization of both renin and pseudorenin while crude substrate served to localize and identify the renin. In addition, assays at pH 7.5 and 5.0 served to identify further the two activities, since the pH optimum for renin is approx 7.5 while that of pseudorenin is much lower.

Fig. 1 shows the separation of 1 unit of crude human renin on a hemoglobin-Sepharose affinity column. Under these conditions 60% of the protein passed directly through the column, whereas 40% was retained, but could be removed

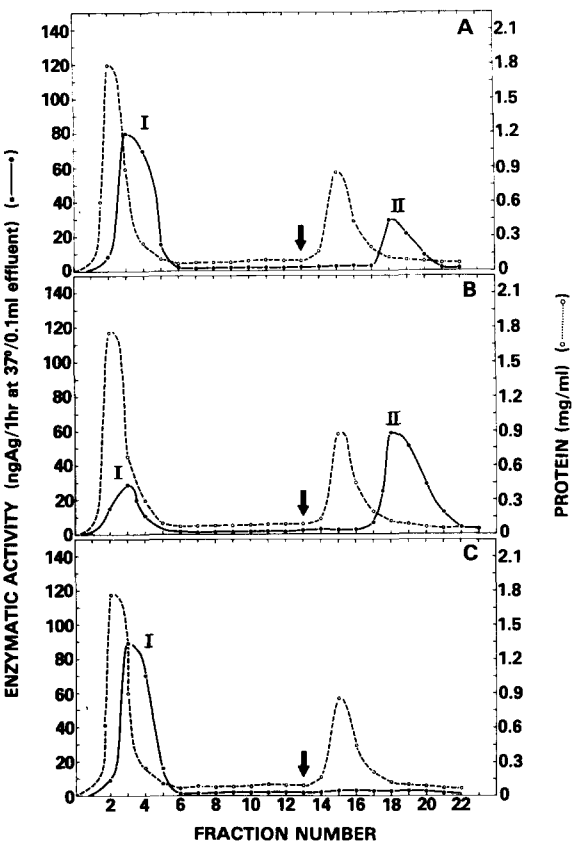

Fig. 1. Hemoglobin-Sepharose-2B chromatography of 1 unit of crude human renin. The starting buffer was 1 M sodium chloride, 0.1 M sodium acetate, pH 3.5. At the point marked with an arrow, column bound proteins (○- - - - -○) were eluted with 0.1 M Tris buffer containing 1.0 M sodium chloride, pH 6.5. A. Enzyme activities (•——•) were determined by radioimmunoassay for angiotensin I after incubation at pH 7.5 with purified hog protein renin substrate. Note that peak I contains more of the angiotensin I forming activity. B. Enzymatic activity was monitored by incubation of enzyme with purified hog protein substrate at pH 5.0. Note that at this pH most of the activity was in Peak II. C. Enzyme activity was monitored with crude hog protein renin substrate. Only a single peak of activity (I) was seen.

by increasing the pH of the elution buffer to 6.5. In the first part of this experiment (A), assays at pH 7.5 showed two peaks of activity (I and II). The activity of Peak I was higher than that of Peak II. The fractions were then assayed for enzyme activity at pH 5.0 (B). Under these conditions, the activity of Peak II was greater than that of Peak I. Based on these results it is suggested that Peak I contained renin and Peak II contained pseudorenin. When the column fractions were monitored using the labeled polymeric substrate assay [12], two peaks were observed, which corresponded to the position of the peaks shown in Fig. 1B. The polymeric substrate is attacked by both renin and pseudorenin. Finally, using crude protein substrate, only a single peak was found which corresponded to Peak I (Fig. 1, C). Since pseudorenin does not form angiotensin I from this substrate, we again identified Peak I as renin and Peak II as pseudorenin.

The fractions under Peak I and Peak II were separately pooled and tested for their proteolytic activity. The renin of Peak I was free of such activity, while Peak II, corresponding to pseudorenin, contained considerable non-specific protease activity. No conclusions can be drawn about the possible identity of pseudorenin with the proteolytic activity, but the behavior of pseudorenin is similar to that of cathepsin D.

It should be emphasized that the presence of a high NaCl concentration during the affinity chromatography was essential to produce this separation, in agreement with the work of Smith and Turk [6]. In an experiment employing an NaCl-free buffer, none of the proteolytic activity was retained on the affinity adsorbent at pH 3.5.

Discussion

Preparation and availability of renin which is free of pseudorenin is desirable for several reasons. Because of the large amounts of pseudorenin activity in plasma and tissue extracts, this enzyme may interfere with renin assays even at neutral pH where pseudorenin is relatively inactive. Furthermore, the relationship of pseudorenin to other proteases is not established. Most readily available renin preparations contain, in addition to pseudorenin, non-specific proteolytic activity ([^{14}C]glycinated hemoglobin as substrate) which is due not to renin itself but to the presence of contaminating proteases (unpublished data). These impure preparations may generate peptides other than angiotensin which may interfere in the various assays for renin. The technique described here readily provides renin which is free of both pseudorenin and protease activity.

Purification of human renal renin has in the past been difficult. Removal of pseudorenin has been somewhat tedious, but probably more important has been the problem of progressive instability of renin during its purification, apparently due to destruction of the renin by contaminating proteases. This difficulty has been overcome during purification of hog renin by the use of a pepstatin affinity-column, ion-exchange and gel filtration column chromatography, and the use of protease inhibitors [4], but complete purification of human renin has remained complicated by enzyme instability as the purification has progressed. Similar problems were encountered during purification of both cathepsin D [6] and RNA polymerase [13]. In those instances, use of hemoglobin-Sepharose-2B affinity columns for elimination of non-specific

proteases allowed isolation of the desired enzyme in stable form. We would expect similar usefulness for this approach in future attempts to isolate human renin.

Note added in proof (Received February 23rd, 1978)

Since this paper was submitted the pseudorenin of spleen has been identified as cathepsin D (Johnson, R.L. and Poisner, A.M. (1977) Biochem. Pharmacol. 26, 2237—2240).

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