Biochimica et Biophysica Acta, 445 (1976) 158—160

BBA 67874

AFFINITY CHROMATOGRAPHY OF ANGIOTENSIN I-CONVERTING ENZYME FROM RABBIT LUNG USING HIPPURYLHISTIDYLLEUCYL-OH

KAZUTAKA NISHIMURA, KUNIO HIWADA, EINOSUKE UEDA and TATSUO KOKUBU

The 2nd Department of Internal Medicine, Ehime University School of Medicine, Shigenobu, Onsen-gun, 791-02 Ehime (Japan)

(Received January 13th, 1976)

Summary

Approximately 50-fold purification of angiotensin I-converting enzyme (Peptidyldipeptide hydrolase, EC 3.4.15.1) from rabbit lung was achieved by affinity chromatography using the synthetic substrate Hippuryl-His-Leu-OH. The specific activity of the enzyme was increased from 0.044 units/mg protein to 1.911 units/mg protein for Hippuryl-His-Leu-OH and from 0.33 nmol/min per mg protein to 13.8 nmol/min per mg protein for angiotensin I.

Angiotensin I-converting enzyme (Peptidyldipeptide hydrolase, EC 3.4.15.1) converts angiotensin I to angiotensin II by releasing the C-terminal sequence residue (His-Leu-OH) of the decapeptides. This enzyme was first isolated from horse plasma [1]. Ng and Vane pointed out the significance of the lung on the metabolism of angiotensin I in vivo [2], and Cushman and Cheung reported that high activity of the enzyme existed in the lung [3]. On the other hand, the enzyme is thought to be capable of inactivating bradykinin and to be the same enzyme as kininase II [4—6]. The characteristics of the physiological and biological function of the enzyme in the lung has not been clearly established. We have therefore attempted to purify the enzyme from rabbit lung by affinity chromatography using the synthetic substrate Hippuryl-His-Leu-OH.

Methods and Results

Synthetic substrate Hippuryl-His-Leu-OH was coupled to agarose via its free carboxyl group according to method described by Cuatrecasas [7].

Step 1. Sepharose 4B (10 ml of the wet gel) was activated by CNBr (1 g), maintaining pH 11 with 2 M NaOH at 20°C. CNBr-acitvated Sepharose 4B was washed immediately with 0.1 M borate buffer (300 ml), pH 9.0, at 0°C and mixed with 0.5 M hexamethylenediamine in the same buffer (10 ml) at 4°C for 24 h.

Step 2. The coupled Sepharose 4B (10 ml) was washed with dioxane (300 ml) and incubated with 100 μmol Hippuryl-His-Leu-OH (obtained from the Institute for Protein Research, Osaka Univ., Osaka, Japan) in dimethylformamide (5 ml) and dioxane (5 ml) in the presence of dicyclohexylcarbodiimide (2 g) at room temperature for 24 h. The coupling of the peptide to the gel was carried out with gentle use of a magnetic stirrer. The gel was washed with 500 ml of ethanol, dioxane, dimethylformamide and then water to remove trapped Hippuryl-His-Leu-OH. 1 ml of the wet gel was found to contain 5.0 μmol of covalently bound Hippuryl-His-Leu-OH, which was determined by amino acid analysis of an acid hydrolysate of the gel.

Enzyme assays

Angiotensin I-converting enzyme assay was performed by the spectrophotometric method of Cushman and Cheung [8]. One unit of the enzyme activity was defined as that amount of enzyme which hydrolyzed 1 μmol Hippuryl-His-Leu-OH per min at 37°C under the conditions described by them. Next, the activity of angiotensin I-converting enzyme was determined by using angiotensin I as substrate. The reaction mixture, contained 0.4 ml of 100 mM potassium phosphate buffer, pH 7.8, 0.5 ml of 5 μM angiotensin I in saline, 0.1 ml of the enzyme solution and a few drops of 0.27 M diisopropylfuruolophosphate, was incubated at 37°C for 10 min and stopped by boiling for 5 min. The angiotensin II formed was assayed in isolated rat uterus [6].

Protein concentration was determined by the method of the Lowry et al. [9].

Fresh rabbit lungs (30 g) were chopped into small pieces and suspended in 120 ml of 20 mM potassium phosphate buffer, pH 7.8, containing 0.25 M sucrose. The suspension was homogenized in a Waring blendor for 4 min and centrifuged for 20 min at 700 × *g*. The supernatant was centrifuged for 90 min at 78 000 × *g*. The pellet was dissolved in 90 ml of 10 mM potassium phosphate

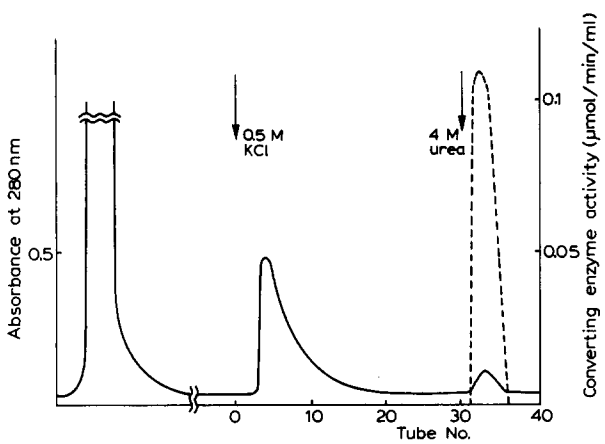

Fig. 1. Hippuryl-His-Leu-Sepharose column chromatography (1.6 × 3 cm) of angiotensin I-converting enzyme from rabbit lung. Sample applied: 8.2 mg protein, specific activity 0.044 units/mg protein. 1 unit = 1 μmol hippuric acid/min/ml. Fraction volume: 3.25 ml. Solid line: absorbance at 280 nm. Broken line: enzyme activity. The starting buffer was 10 mM potassium phosphate, pH 6.8.

buffer, pH 7.8. The dissolved material was stirred with a magnetic stirrer for 1 h in the presence of 2% Triton X-100 and 2 M urea, and then centrifuged for 90 min at 78 000 × *g*. The supernatant was concentrated to 50 ml with Amicon PM 10 filter and dialysed overnight against 10 mM potassium phosphate buffer, pH 6.8. The specific activity of the dialysed solution was 0.044 units/mg protein for Hippuryl-His-Leu-OH and 0.33 nmol/min/mg protein for angiotensin I (extract of 2% Triton X-100 and 2 M urea). This material was used for the affinity column chromatography.

The affinity column (1.6 × 3 cm) had been equilibrated with 10 mM potassium phosphate buffer, pH 6.8. After the application of the sample (8.2 mg protein), the column was washed with 500 ml of the same buffer to remove detergent and with 0.5 M KCl in this buffer to exhaust proteins into eluate. The fraction containing the angiotensin I-converting enzyme was eluted with 4 M urea whereupon the specific activity was increased to 1911 units/mg protein for Hippuryl-His-Leu-OH and to 13.8 nmol/min/mg protein for angiotensin I. Recovery of the enzyme activity for Hippuryl-His-Leu-OH was approximately 80%. The elution pattern of the enzyme is indicated in Fig. 1.

Discussion

There have been several reports of the affinity chromatography of renin using renin inhibitor. But the affinity chromatography of angiotensin I-converting enzyme has not been reported. We tried to carry out affinity chromatography using the synthetic substrate Hippuryl-His-Leu-OH. An approximately 50-fold purification was achieved by this method. Das and Soffer reported purification of the enzyme using affinity chromatography on ricin agglutinin [10]. But their affinity column was specific for galactose residue and not for angiotensin I-converting enzyme. The affinity chromatography of our method was found to be a good procedure for purification of angiotensin I-converting enzyme. Our data indicate that this column will facilitate purification work and contribute to making clear the mechanism of the enzyme-substrate interaction.

Acknowledgement

This work was supported in part by grant No. 057219 from Ministry of Education.

References

1 Skeggs, Jr., L.T., Kahn, J.R. and Shumway, N.P. (1956) J. Exp. Med. 103, 295–299
2 Ng, K.K.F. and Vane, J.R. (1967) Nature 216, 762–766
3 Cushman, D.W. and Cheung, H.S. (1972) in Hypertension, (Genest, J. and Koiw, E., eds.), pp. 532–541, Springer-Verlag, Berlin
4 Yang, H.Y.T., Erdös, E.G. and Levin, Y. (1970) Biochim. Biophys. Acta 214, 374–376
5 Yang, H.Y.T., Erdös, E.G. and Levin, Y. (1971) J. Pharmacol. Exp. Ther. 117, 291–300
6 Ueda, E., Akutsu, H., Kokubu, T. and Yamamura, Y. (1971) Jap. Circul. J. 35, 801–806
7 Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065
8 Cushman, D.W. and Cheung, H.S. (1971) Biochem. Pharmacol. 20, 1637–1648
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
10 Das, M. and Soffer, R.L. (1975) J. Biol. Chem. 250, 6762–6768