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Note

Rapid purification of human placental angiotensin I converting enzyme by captopril affinity chromatography

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Angiotensin I converting enzyme (ACE, peptidyldipeptide hydrolase, E.C. 3.4.15.1) converts angiotensin I to angiotensin II by releasing the C-terminal sequence residue (His-Leu) of the decapeptide, and also degrades the vasodepressor peptide bradykinin [1].

Captopril, D-(3-mercapto-2-methylpropanoyl)-L-proline, is a potent inhibitor of ACE with a k_i value of 5.7 n*M* [2]. Since Gavaras et al. [3] demonstrated that captopril reduces blood pressure in severely hypertensive patients, treatment of hypertension by regulating the angiotensin concentration by inhibitors of ACE has been under investigation. We have attempted to use this drug as an affinity ligand for the purification of ACE from human placenta.

MATERIALS AND METHODS

Hip-His-Leu (hippurylhistidylleucine) was purchased from the Peptide Institute, Osaka, Japan. Captopril was kindly supplied by Sankyo, Tokyo, Japan.

Preparation of the affinity column

Captopril (48 mg) was esterified with 160 mg of N-hydroxysuccinimide in the presence of 160 mg of N-ethyl-N'-carbodiimide hydrochloride at 4°C for 16 h. AH-Sepharose 4B (4 g suction-dry weight) [4], after repeated washing with 0.5 *M* sodium chloride and distilled water, was added to a solution of the activated captopril, and coupling of the peptide to the gel was carried out at

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room temperature for 22 h with gentle stirring under exclusion of atmospheric moisture. The gel was washed with distilled water and then with 20 m*M* potassium phosphate buffer (pH 7.8); 1 ml of the wet gel was found to contain 13.9 μmol of covalently bound captopril.

Purification of ACE from human placenta

Fresh human placenta (200 g) after normal delivery was chopped into small pieces and suspended in 400 ml of 20 m*M* potassium phosphate buffer (pH 7.8) containing 0.25 *M* sucrose. The suspension was homogenized for 30 min with a Polytron homogenizer at setting 7. The resulting homogenate was centrifuged at 700 *g* for 30 min. The supernatant was adjusted to pH 5.2 with 0.1 *M* acetic acid, stirred for 5 min and centrifuged at 15 000 *g* for 30 min. The pellet was suspended with 50 ml of 20 m*M* potassium phosphate buffer (pH 7.8) and adjusted to pH 7.8 with 1 *M* sodium hydroxide. This solution was incubated with a solution of trypsin (1 mg per 500 mg of protein) containing 1 m*M* calcium chloride for 120 min at 37°C and then centrifuged at 15 000 *g*. The supernatant was adjusted to pH 7.8 with 1 *M* sodium hydroxide, dialysed overnight against 2000 ml of 20 m*M* potassium phosphate buffer (pH 7.8) and applied to the captopril affinity gel (1.6 × 16 cm) previously equilibrated with 20 m*M* potassium phosphate buffer (pH 7.8). The column was washed with the same buffer and eluted with a 200-ml linear gradient of sodium chloride (0—0.5 *M*).

Enzyme assay

ACE activity was measured by high-performance liquid chromatography according to the method of Horiuchi et al. [5] using Hip-His-Leu as a substrate. One unit of activity is defined as the amount of enzyme catalysing the release of 1 nmol of hippuric acid from Hip-His-Leu per min at 37°C. Protein was determined according to the method of Lowry et al. [6] with bovine serum albumin as a standard. Polyacrylamide slab gel electrophoresis was performed by the method of Davis [7].

TABLE I

PURIFICATION OF HUMAN PLACENTAL ANGIOTENSIN I CONVERTING ENZYME

Purification step	Total activity (nmol/min)	Total protein (mg)	Specific activity (nmol/min/mg)	Purification (*n*-fold)	Activity recovered (%)
The supernatant of homogenate centrifuged at 700 *g*	2120	3320	0.63	1	100
Acidification, the pellet centrifuged at 15 000 *g*	310	760	0.40	0.6	15
Trypsin treatment	475	135	3.52	5.6	22
Affinity chromatography	305	$108 \cdot 10^{-3}$	2824	4482	14

RESULTS AND DISCUSSION

As summarized in Table I, a 4482-fold purification was achieved to obtain a purified preparation of ACE from the human placenta with an overall yield of 14%. An approximately 800-fold purification achieved by the captopril affinity chromatography facilitated the present purification work (Fig. 1). The major ACE peak fractions obtained by this chromatography gave a single band on electrophoresis in polyacrylamide gel (Fig. 2).

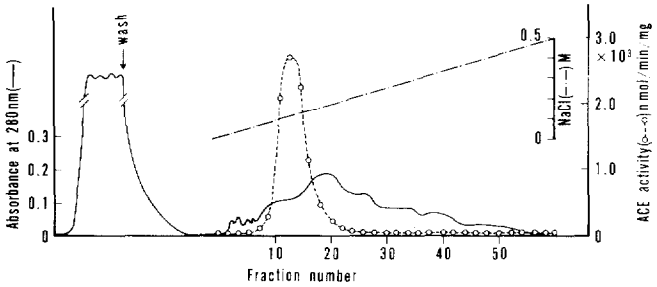

Fig. 1. Purification of angiotensin I converting enzyme on captopril affinity chromatography. (—) Distribution of proteins monitored by UV absorbance at 280 nm; (- - -) distribution of angiotensin I converting enzyme monitored by enzymatic activity. The flow-rate was 0.3 ml/min and the fraction volume was 3.25 ml.

Although affinity gels using the immunoadsorption technique [8] and potent inhibitors such as D- and L-cypteinyl-L-proline [9] have been used to purify human serum ACE successfully, relatively harsh elution conditions, such as 2 *M* magnesium chloride or 3 *M* urea, may have resulted in preparations with lower specific activity. In the present study, such conditions were avoided by using a low concentration of sodium chloride (0.5 *M*) to elute the enzyme from the affinity gel (Fig. 1). Another type of affinity column was recently developed by El-Dorry et al. [10], utilizing a potent inhibitor of ACE, N-α-[1-(*S*)-carboxy-3-phenylpropyl]-L-lysyl-L-proline, as affinity ligand.

ACE was first isolated from horse plasma [11]. Since the work of Ng and Vane [12] implicating the pulmonary vasculature as a major site of conversion of angiotensin I to angiotensin II, attention had been focused on the properties of the lung enzyme [13, 14]. We could purify ACE from human placenta by affinity chromatography using captopril, the orally active antihypertensive agent and potent inhibitor of ACE.

The physiological role of the ACE in various human tissues is not yet well understood. In the field of obstetrics and perinatal medicine, abundant evidence points to the importance of the renin—angiotensin and kallikrein—kinin systems in the physiological adjustment of circulation of a fetus in the feto-placental unit [15, 16]. The purified enzyme from human placenta by the present method could be used in the elucidation of the physiological significance of human ACE.

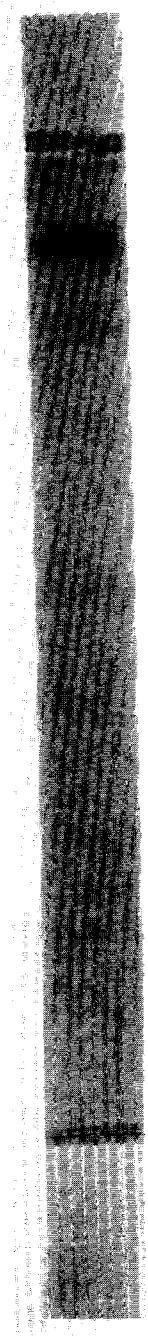

Fig. 2. Slab gel electrophoresis of the human placental angiotensin I converting enzyme in the major peak fractions of Fig. 1. The gel was stained for protein with Coomassie Brilliant Blue.

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