PURIFICATION AND STUDY OF THE PHYSIOCOCHEMICAL PROPERTIES OF ANGIOTENSIN-CONVERTING ENZYME FROM HUMAN LIVER

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Angiotensin-converting enzyme (ACE) catalyzes the formation of vasoactive angiotensin II and inactivation of bradykinin, with removal of the C-terminal dipeptides [1, 14, 15], and it is thus the key enzyme of the renin-angiotensin and kallikrein-kinin systems [2]. The great physiological importance of this enzyme in blood pressure regulation and the use of its inhibitors in the treatment of hypertension served as the motivation for an intensive study of ACE.

The enzymic activity of ACE is found in various animal tissues [3, 10], including human tissues [7, 10]. Isolation of ACE has now been described from sources such as the kidneys, lungs, brain, seminal fluid, and blood plasma [11].

In this investigation the human liver was used for the first time as the source of ACE. ACE was isolated from the tissue of this organ by chromatofocusing and some of its physicochemical parameters were studied, so that it could be compared to some extent with ACE isolated from other organs.

EXPERIMENTAL METHOD

ACE activity was measured fluorometrically in 50 mM Tris-HCl, pH 8.3, containing 0.4 M NaCl and 2.5 mM hippuryl-histidyl-leucine (HHL), or in 50 mM Tris-HCl, pH 7.3, containing 0.2 M NaCl and 0.5 mM N-benzyloxycarbonyl-phenylalanyl-histidyl-leucine (BPHL) [12]. Incubation was carried out at 37°C for 15 min. Protein was determined by Lowry's method [8]. Electrophoresis was carried out in 7.5% polyacrylamide gel in the presence of 0.1% sodium dodecylsulfate [5].

EXPERIMENTAL RESULTS

ACE plays an important role in the diagnosis of liver diseases [9]. However, there are no data whatever in the literature of ACE from the liver.

The results of isolation of ACE from human liver are given in Table 1. All procedures were carried out at 4°C. For this purpose 1.5 kg of cadaveric liver was homogenized in 9 volumes of 50 mM phosphate buffer, pH 8.3. The resulting homogenate was centrifuged at 1000g for 10 min. The supernatant was centrifuged at 21,000g for 60 min. The residue was resuspended in 650 ml of the original buffer and sedimented by recentrifugation. ACE was converted into the soluble state by suspending 650 ml of the residue in 350 ml of 3 mM $CaCl_2$ with the addition of 500 mg of trypsin. Proteolysis was carried out for 1 h at 37°C, after which the reaction was stopped by addition of 500 mg of soy trypsin inhibitor. Soluble ACE was isolated from the membrane fraction by centrifugation at 21,000g for 80 min. The supernatant was dialyzed against distilled water and ion-exchange chromatography was carried out on DEAE-cellulose, equilibrated with 10 mM phosphate buffer, pH 7.8. The enzyme was eluted by the same buffer, containing 0.2 M NaCl. Rechromatography on DEAE-cellulose was carried out in a column containing a linear NaCl gradient from 0 to 0.2 M. For further purification Sephadex S-300 was used. Fractions containing ACE were dialyzed against 0.025 M histidine-HCl, pH 6.2, and

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TABLE 1. Isolation of ACE from Human Liver

Procedure	Specific activity U/mg protein	Total activity, U	Degree of purification	Purification yield, %
Homogenate	$1,1 \cdot 10^{-3}$	275	1	100
Membrane fraction	$5,0 \cdot 10^{-3}$	250	5	91
Proteolysis	$3,9 \cdot 10^{-2}$	272	35	99
DEAE-cellulose (without column)	$1,1 \cdot 10^{-1}$	187	100	68
DEAE-cellulose (using column)	$6,2 \cdot 10^{-1}$	102	563	37
Sephacryl S-300	1,8	91	1636	33
Chromatofocusing	10	60	9090	22

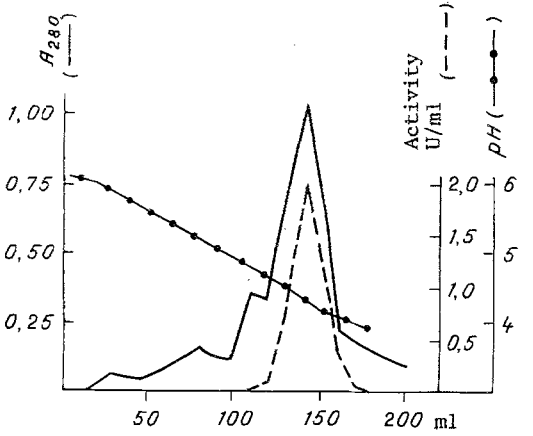

Fig. 1. Chromatofocusing of ACE on PBI-94 (9 × 250 mm), equilibrated with 0.025 M histidine-HCl buffer, pH 6.2. Elution by polybuffer 74 (dilution 1:8), pH 4.0; flow rate 14 ml/h.

applied to ion-exchange resin PBI-94, and subjected to chromatofocusing within the pH range from 6.0 to 4.0 (Fig. 1). The ACE thus obtained was separated from components of the polybuffer 74 on Sephadex G-75, concentrated, and kept at −40°C.

By adding a stage of chromatofocusing to the traditional scheme of enzyme isolation, it was possible to purify ACE from human liver by more than 9000 times and to obtain ACE with specific acitivty of 10 U/mg protein. The purity of the enzyme thus obtained was monitored under denaturing conditions. The position of the protein band corresponded to a protein with mol. wt. of 150 kilodaltons (kD). This value is close to those recorded for ACE from the lungs and kidneys [11] but differs from mol. wt. of the testicular enzyme, namely 100 kD [13].

The most important physicochemical parameters were determined for hepatic ACE. The isoelectric point of ACE from human liver, determined by chromatofocusing, was found to be 4.2-4.3. This value of pI indicates that ACE from the liver is more acid in character than the renal and pulmonary enzymes [11]. The lower value of pI of ACE isolated from the liver is evidently due to the higher content of sialic acids in the carbohydrate moiety of the glycoprotein.

During the study of enzymic hydrolysis of HHL and BPHL in the presence of ACE from human liver, the Michaelis constants (K_m) were determined: their values were 5 and 0.1 mM, respectively, within the limits of values obtained previously [4]. The peptidase from human liver is effectively inhibited by the specific ACE inhibitor SQ20881, with IC_{50} of $1.8 \cdot 10^{-8}$ M. Comparison of the value obtained with IC_{50} for interaction between SQ20881 and ACE from human lungs showed that both enzymes are inhibited equally effectively [6].

By using the suggested purification scheme, it was thus possible to isolate from human liver an ACE whose molecular and kinetic parameters are almost indistinguishable, except for isoelectric point, from the analogous values for ACE from other sources.

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DIAGNOSTIC VALUE OF LEUCINE-AMINOTRANSFERASE ASSAY IN ACUTE PANCREATITIS

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Leucine aminotransferase (LAT), which catalyzes the reaction transferring an amino group from leucine to α-ketoglutaric acid with the formation of α-ketoisocaproic and glutamic acids, is found in all tissues of the human and animal body and is located both in the cytoplasm and in the subcellular organelles [4-6]. High activity of this enzyme in the pancreas, salivary glands, and lactating mammary glands points to its important role in the function and metabolism of these organs [4, 7]. These facts suggest that destructive diseases of the pancreas may be accompanied by significant changes in LAT activity both in that organ and in the blood.

The aim of this investigation was to determine the differential diagnostic value of investigation of LAT activity in the blood serum and peritoneal exudate in different forms of acute pancreatitis (serous and destructive pancreatitis).

EXPERIMENTAL METHOD

LAT activity was determined in the blood serum of 10 clinically healthy individuals (blood donors) and in the serum and peritoneal exudate (if present) of 20 patients with acute pancreatitis. LAT activity was determined in the pancreatic tissue and blood serum of intact (15) rats and rats undergoing a mock operation (24), and in the blood serum and peritoneal exudate of rats with pancreatic necrosis (40 animals). Experimental subtotal pancreatic necrosis was induced by cooling the splenic part of the pancreas with ethyl chloride [3], and serous pancreatitis was induced by interference with the drainage of pancreatic juice [2]. LAT activity was determined in the cytoplasmic (soluble) fraction of pancreatic tissue obtained by differential centrifugation of a 10% tissue homogenate at 25,000g. The

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