MOLECULAR FORMS OF ACID BRAIN PROTEINASES

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Acid proteinases of the brain were isolated by gel filtration on a column with Sephadex G-100. Three peaks of enzyme activity were found. Cathepsin D with high molecular weight (over 150,000) is solubilized from brain tissues by 0.1% Triton X-100. Cathepsin D with a molecular weight of 59,000 is solubilized only by a 0.2% solution of the detergent. Low-molecular-weight (20,000) cathepsin D is a soluble cytoplasmic enzyme. The results are regarded as proof of the heterogeneity of molecular forms of acid proteinases in the cerebral cortex, corresponding to the strength of the bond between the enzymes and subcellular structure of brain tissue.

KEY WORDS: brain; acid proteinases – fractionation; cathepsin D; molecular forms.

The writers previously studied the behavior of acid brain proteinases during solubilization with Triton X-100, as shown by the presence of S-shaped and linear portions of the solubilization curve, evidence of of the existence of several forms of bonds between enzyme and nerve tissue membranes [6].

The investigation described below was carried out to study differences in molecular forms of acid proteinases possessing cathepsin type D activity and solubilized by 0.1% (S-shaped portion of the curve) and 0.2% (S-shaped + linear portions of the solubilization curve) solutions of nonpolar detergent respectively.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature cats. Homogenates were prepared from the tissue of the cerebral cortex and soluble and Triton fractions were obtained as described previously [6]. During preparation of the Triton extracts (0.1 and 0.2%) the soluble fraction was not separated beforehand.

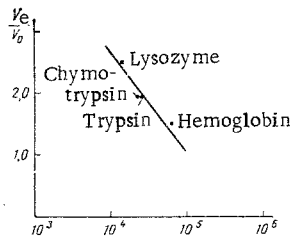

Fig. 1. Elution volume as a function of molecular weight of protein. Abscissa, molecular weight (logarithmic scale); ordinate, ratio between elution volume of protein and free volume (V_e/V_0).

The extracts, in a volume of 0.2 ml, were applied to a column with Sephadex G-100 (17 × 0.8 cm), equilibrated with 0.85% NaCl. Protein was eluted at the rate of 18-20 ml/h and 0.2-ml samples were collected in tubes containing incubation mixture for determination of acid proteinase activity. Proteinase activity was determined by Anson's method [7], using hemoglobin denatured by urea [5]. The incubation mixture, in a final volume of 0.7 ml, contained 1% hemoglobin, 0.135 M Na-acetate buffer, pH 3.8, and 2.5 M urea. After incubation (30 min) the reaction was stopped by the addition of a cold 5% solution of TCA. The quantity of hemoglobin breakdown products soluble in TCA was determined spectrophotometrically at 280 nm. The molecular weight of the enzymes was determined from a calibration curve (Fig. 1). The following substances were used to calibrate the Sephadex column: lysozyme (14,400), chymotrypsin (22,500) trypsin (23,800), hemoglobin (64,500), and blue dextran. The protein content in the fractions was determined spectrophotometrically

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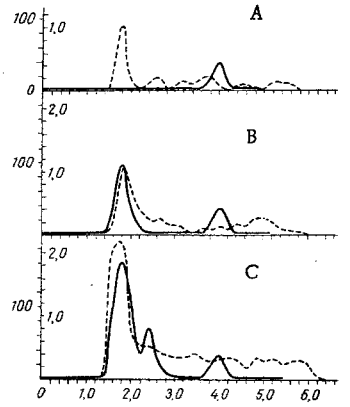

Fig. 2. Elution profile of protein and activity of brain acid phosphatases: A) soluble fraction of cerebral cortex; B and C) 0.1% and 0.2% Triton extract of cerebral cortex, respectively. Continuous line – enzyme activity; broken line – distribution of protein among fractions. Abscissa, volume of eluate (in ml); ordinate: on the left – activity of acid proteinases (in μg tyrosine removed from hemoglobin in 1 h at 37°C and pH 3.8 calculated for 1 ml eluate), on the right – protein content (in mg/ml eluate).

TABLE 1. Gel-Chromatographic Analysis of Acid Proteinases from Gray Matter of the Cat Cerebral Cortex (M±m)

Conditions of extraction and number of experiments (n)	Initial activity of extract	I peak of activity		II peak of activity		III peak of activity	
		total activity of peak (8th-11th fraction)	mol. wt.	total activity of peak (12th-14th fraction)	mol. wt.	total activity of peak (19th-21st fraction)	mol. wt.
0,32 M sucrose (n=7)	40,0±5,9	0	–	0	–	70,0±9,6	20 900±1 100
0,1% Triton X-100 (n=7)	204,0±16,2	184,3±11,2	>150 000	0	–	70,9±5,7	19 000±1 300
0,2% Triton X-100 (n=12)	288,0±12,3	375,0±40,8	>150 000	101,0±10,0	59 000±3 300	66,0±9,4	19 900±1 400

Legend. Activity of acid proteinases expressed in μg tyrosine removed from hemoglobin during incubation for 1 h at 37°C and pH 3.8, calculated for 1 ml of eluate.

from absorption at 280 and 260 nm and calculated from a nomogram [2]. The numerical results were subjected to statistical analysis [4].

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiments to fractionate acid proteinases of the cat cerebral cortex by gel filtration (Table 1; Fig. 2) show that different types of bond linking the acid proteinases with the brain tissue [6] correspond to different molecular forms of the enzymes. The free enzyme (not bound with subcellular structures) in a proteinase with low molecular weight which, in the procedures of fractionation and purification of cathepsin D usually used [3, 11], was evidently discarded as a minor component relative to the other molecular forms. At the present time low-molecular-weight cathepsins (F and G) have been found only in leukocytes [9]. A 0.1% solution of Triton X-100 solubilized the high-molecular-weight (150,000) acid proteinase. By the action of a 0.2% solution of the detergent, an enzyme with cathepsin D activity (mol. wt. 59,000) was solubilized. By its molecular dimensions this was an acid proteinase possibly analogous to the enzyme that has been isolated, purified, and identified in various tissues as cathepsin D [8, 10, 11]. A 0.2% solution of the detergent (but not the 0.1%) also solubilized the high-molecular-weight form of the acid proteinases. It is a very interesting fact that the soluble (free) enzyme was eluted as a separate (III) peak. This suggests that the presence of a free enzyme with cathepsin D activity in brain tissue is not an artifact caused by the homogenization and centrifugation procedures.

The results examined above thus indicate the existence of several molecular forms of acid proteinases in the cerebral cortex with cathepsin D activity, and differing in the character of their bond with the subcellular structures of brain tissue.

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