SOLUBILIZATION OF ACID PROTEINASES BY THE DETERGENT TRITON X-100 IN FUNCTIONALLY DIFFERENT CNS STRUCTURES

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The character of solubilization of acid proteinases by Triton X-100 was found to differ in functionally different structures of the cat CNS. The initial S-shaped segments of the solubilization curves coincide for tissues of the gray and white matter of the cerebral hemispheres; the subsequent linear segments differ in their gradients. The existence of several forms of binding of acid proteinases in the brain tissues is postulated.

Functionally different structures of the CNS differ in their acid proteinase activity [2]. The writers showed previously that the gray and white matter of the cerebral hemispheres also differ in their content of free cathepsins, not bound with subcellular structures [3]. For instance, activity of the free enzymes in the gray matter of the cerebral hemispheres amounts to less than 10% of their total activity, whereas in the white matter almost one-third of the acid proteinase activity is in the free form, not bound with subcellular structures. These differences are undoubtedly connected with differences in protein catabolism in the structures of the gray and white matter.

Data on the solubilization of acid proteinases by the nonionic detergent Triton X-100 in the gray and white matter of the cat cerebral hemispheres are described below.

EXPERIMENTAL METHOD

Sexually mature cats were used. After decapitation the brain was quickly removed and placed in cold 0.25 M sucrose solution. All operations with the brain were carried out at 0-3°C. After removal of the meninges the cerebral hemispheres were divided into gray and white matter and 10% homogenates (from samples weighing 1-1.2 g) were prepared in a glass homogenizer with Teflon pestle (1200 rpm; 4-6 runs). The homogenization medium described previously [3], containing 0.32 M sucrose and 0.01 M $MgCl_2$ (ph 5.0), was used. The total acid proteinase activity in the homogenate was determined. The homogenates were centrifuged for 60 min at 22,000 g. Activity of free cathepsins was determined in the supernatant (soluble fraction). The residue was resuspended in the isolation medium and again centrifuged at 22,000 g for 60

TABLE 1. Activity of Acid Proteinases in Various Brain Fractions (in μg tyrosine split from hemoglobin during incubation for 1 h at 37°C and pH 3.8; M ± m)

Portion of cerebral hemisphere	Soluble fraction	Washings fraction	Residue	Homogenate	Percent of activity of free enzyme
Gray matter	0,69±0,05	3,37±0,04	11,7±0,5	11,9±0,6	5,6±0,5
White matter	1,11±0,17	0,35±0,06	3,3±0,2	5,0±0,1	23,8±1,6

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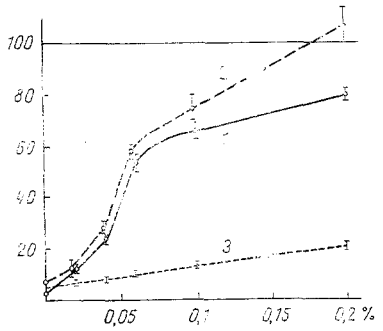

Fig. 1. Solubilization of acid proteinases and protein from the fraction of subcellular brain particles. Abscissa, concentration of Triton X-100 (in % by volume); ordinate, activity of solubilized acid proteinases (in % of total activity of fraction); 1,2) solubilization of acid proteinases of gray and white matter of cerebral hemispheres respectively; 3) solubilization curve of protein of the cerebral cortex.

min. The resulting soluble fraction was described as the "washings fraction." The washed residue contained the fraction of subcellular structures and was used to determine the activity of bound cathepsin.

The residue was resuspended again in homogenization medium and Triton X-100 was added to final detergent concentrations of 0-0.2% (by volume). The samples were thoroughly stirred (20 min, 0°C) and centrifuged at 22,000 g for 60 min. Activity of the cathepsins solubilized from the subcellular structures was then determined.

Activity of the acid proteinases in the samples was determined by Anson's method [4], using hemoglobin denatured with urea [3]. The incubation mixture in a final volume of 2 ml contained: hemoglobin 1%, acetate buffer (pH 3.8) 0.135 mole, urea 2.5 moles, $MgCl_2$ 0.0025 mole. The concentration of detergent in the samples in which the activity of the cathepsins solubilized by Triton was determined was adjusted to a final value of 0.02%. Triton X-100, in the above concentration, has no appreciable effect on enzyme activity (the percentage of activity of acid proteinases in homogenates treated with detergent, relative to the untreated, was 95.5 ± 2.6).

After incubation for 1 h, the experimental and control samples were treated with 3 ml cold 5% TCA solution. The concentration of TCA-soluble hemoglobin breakdown products was determined spectrophotometrically (SF-4A) from the absorption at 280 nm. Activity of the enzymes was expressed in micrograms tyrosine split off during incubation for 1 h at 37°C and pH 3.8, calculated per milligram fresh tissue. The protein concentration in the samples was determined by Lowry's method [5]. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results (Table 1) show that practically all the acid proteinase activity in the gray matter of the cerebral hemispheres was bound with the intracellular structures. Nearly 25% of the cathepsin activity in the white matter was in the soluble form. The washings fractions contained only small quantities of enzyme activity.

The curve of liberation of acid proteinases from the fractions of the subcellular structures (Fig. 1) was identical for the gray and white matter in the initial part (50% solubilization) and evidently reflects the pattern of solubilization of the lysosomal acid proteinases [6]. The S-shaped character of the curve, with a segment showing a sharp rise (0.04-0.06% Triton X-100 solution) possibly reflects the fact that the acid proteinases in the lysosomes are in the free form, and the only factor limiting their liberation was the lipoprotein membrane of the lysosomes, destruction of which by the detergent in a certain concentration led to the sudden liberation of a large quantity of enzymes. The properties of the lysosomes of the gray and white matter, judging from coincidence between the solubilization curves in their initial part, are evidently identical. The origin of the large quantity of free enzyme in the white matter of the cerebral hemispheres is all the more interesting. This phenomenon cannot be explained by differences in the stability of the lysosomal structures in the tissues of the white and gray matter with the homogenization and centrifugation procedures used.

The segment of the curve corresponding to solubilization of the enzymes with 0.06-0.2% Triton X-100 solution showed that the solubilization of acid proteinases is a linear function of the detergent concentration. Solubilization of protein from the fraction of subcellular particles of the gray matter was a similar linear function throughout its extent. In this case an enzyme bound with the membrane itself was evidently solubilized. In the white matter a 0.2% solution of Triton X-100 led to solubilization of all the enzyme whereas in the gray matter only 70% of the acid proteinase was solubilized.

The results examined above thus point to the existence of many forms of binding of acid proteinases in the brain tissues.

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