ON INACTIVE PRECURSOR OF CATHEPSIN D FROM CHICKEN LIVER

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Summary. Two enzyme preparations (components I and II) were isolated from chicken liver by means of DEAE-cellulose chromatography. The highest proteolytic activity was seen in the zone of comparatively low protein concentration. Storage of the isolated first component in acid medium at pH 3 resulted in considerable increase of its activity, as well as in increase of its ability to bind to diazocarboxyl inhibitor. In the course of this reaction an additional, highly active component and an inactive, low-molecular-weight fraction were formed.

The data obtained in studies on isolation of cathepsin D from chicken liver suggest the existence of an inactive precursor of the enzyme. The preliminary results of these studies are reported herein.

Materials and Methods. Chicken liver cathepsins were obtained from acetone powder in the course of 45 min extraction by 0.005 M citrate buffer at room temperature and pH 5.3. The fraction, precipitating at 33-56% ethanol concentration, was dissolved in citrate buffer and fractionated with acetone; the 44-70% fraction was collected, passed through a column of Sephadex G-100 equilibrated with 0.005 M phosphate buffer, pH 7.3, and subjected to DEAE-cellulose chromatography in the same buffer using stepwise elution by NaCl.

Enzymatic activity was determined by the method of Anson (1). The samples contained 1 ml of 1% hemoglobin solution, pH 3, and 25 ug of the enzyme. Incubation was carried out at 37°C within 30 min. Activity is expressed in the units of optical density of the filtrate after addition of CCl_3COOH (final concentration 5%).

Activation of the first fraction was performed in citrate buffer,

pH 3, at room temperature. Activation time varied from 30 min to 18 hrs.

Reaction of cathepsin with diazoacetylglycine ethyl ester was run in the presence of Cu^{2+} ions, at pH 5 and room temperature (2). Samples, 1 ml each, contained: 1 mg of the enzyme, 250 ug of $Cu(CH_3COO)_2$ and 250 ug of the inhibitor. Before being added to a sample, the inhibitor and $Cu(CH_3COO)_2$ solution were mixed and preincubated for 10 min.

Molecular weights were estimated by means of gel filtration through a Sophadex G-100 column (3).

Results and Discussion. The data on DEAE-cellulose chromatography of the active protein fraction are presented in Fig. 1. Two protein peaks are seen, corresponding to NaCl concentrations of 0.1 M and 0.3 M, respectively. Enzymatic activity measurements showed that the elution curve for proteins did not coincide with the curve of distribution of proteolytic activity. The highest enzymatic activity was seen after the emergence of the first protein peak. Following chromatography on DEAE-cellulose, 3 fractions were collected: the first, emerging at 0.1 M

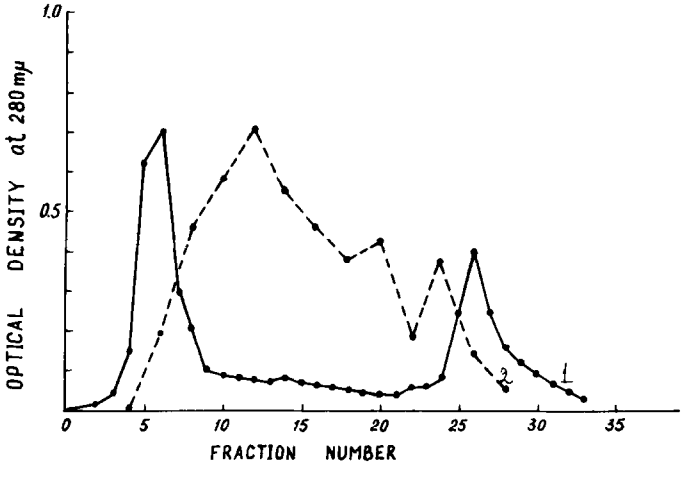

Fig. 1. DEAE-cellulose chromatography of chicken liver cathepsins. Chromatography was carried out in 0.005 M phosphate buffer, pH 7.3. The elution was performed by the buffer containing gradually increasing concentrations of NaCl. Column dimensions were 250 X 18 mm, flow rate - 30 ml per hour. 1 - optical density at 280 nm; 2 - proteolytic activity (in the units of optical density at 280 nm).

NaCl concentration; the second, highly active fraction, emerging in the intermediate zone; and the third, corresponding to 0.3 M NaCl concentration. It should be noted that relative content of individual fractions, as well as their specific activity, varied from one experiment to another, while qualitative pattern of their distribution on DEAE-cellulose remained unchanged.

On storage, and especially upon lyophilization, partial inactivation of the enzyme was observed.

As can be seen from electrophoretic patterns presented in Fig. 2, neither the first, nor the third component were homogeneous. The second

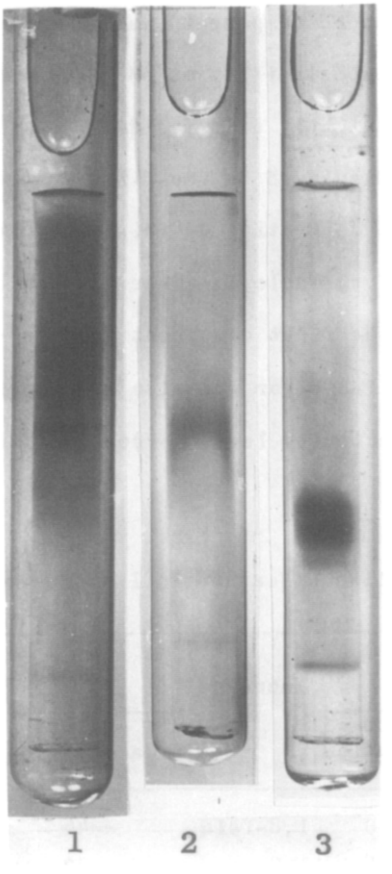

Fig. 2. Polyacrylamide gel electrophoresis of chicken liver cathepsins. Electrophoresis was carried out in Tris-glycine buffer, pH 8.9. Voltage gradient was 5 mA per 1 tube. Time 90 min.

component gave on electrophoregram only one, rather blurred zone, electrophoretic mobility of which corresponded to one of the zones of the first fraction. The third component was characterized by higher electrophoretic mobility.

Molecular weights of both the first and the third component, obtained by chromatography on DEAE-cellulose, were approximately determined and were found to be 45.000-50.000 and 60.000-65.000, respectively.

Studies on inhibition of liver cathepsins by diazocarbonyl inhibitor showed that activity of the first component was inhibited by 16-34%, activity of the second component - by 50-70% and that of the third - by 80-100%.

The enzyme (the second component) was inactivated at pH below 2 and exhibitdd the highest stability at pH 3; it was also found to be stable in neutral and slightly acid medium.

Maximal activity displayed by the first component upon storage at pH 3 suggested possible activation of this component under these conditions. As can be seen from Table I, after incubation within 30 min at pH 3, the activity of the first component was increased 2-5-fold. Its ability to bind to the inhibitor was also increased. Of interest is the fact that the rise in activity level corresponds to the rise in the ex-

Table 1

Activation and inhibition of cathepsin 1 upon storage in acid medium

Exp. No.	Activity		Increase	Inhibition(%)		Increase
	Initial	After activation		Before activation	After activation	
1	0,140	0,250	1,8-fold	44	77	1,75-fold
2	0,275	0,470	1,7 ,,	33	54	1,63 ,,
3	0,095	0,360	3,8 ,,	-	-	-
4	0,05	0,275	5,5 ,,	-	-	-

tent of inhibition. The value of inhibition was similar to that obtained for the second component. It should also be noted that the highest "activation" in acid medium was observed in those cases when the preparations with the lowest initial activity were used. However, in neither of the activation experiments did the activity of the first fraction reach the level of activity of the second component. That is why a series of experiments with prolonged activation (18 hrs) was carried out, using the samples corresponding to that region of the first peak which was characterized by comparatively low proteolytic activity. The results are presented in Fig. 3. After activation and separation of the reaction

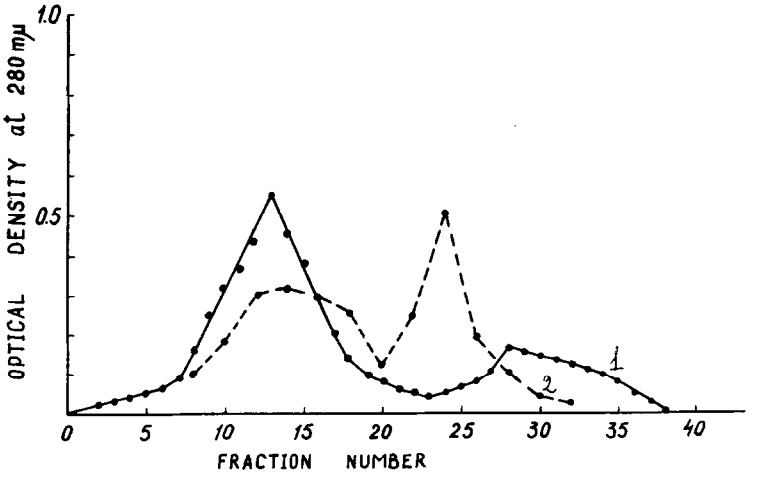

Fig. 3. Filtration of chicken liver cathepsin (the first component) through Sephadex G-100 column after activation in acid medium. Filtration was carried out in 0.005 M phosphate buffer, pH 7.3. Column dimensions were 70 X 1.5 cm, flow rate - 20 ml per hour. 1 - optical density at 280 nm; 2 - proteolytic activity (in the units of optical density at 280 nm).

mixture on Sephadex G-100 column, two components were obtained; the first, active component with a molecular weight of 45.000-50.000, and the second, virtually inactive, with a molecular weight of less than 15.000. Furthermore, after the emergence of the first peak, the formation of a highly active component was observed. The role of the low-molecular-weight component is not yet clear; its addition to the reac-

tion mixture had no effect on the proteolysis. The second active component appeared unstable and was completely inactivated on storage at 5°C within 40 hrs. A more detailed study of this component was impossible at present because of its low stability and limited availability of the material.

Thus, the results of our experiments suggest that chicken liver cathepsins naturally occur in tissues in the form of inactive precursors. Under conditions employed in our isolation procedure the activation of the enzyme apparently proceeds, which manifests itself quite distinctly upon chromatography on DEAE-cellulose. On storage in acid medium the activation proceeds even more rapidly.

Barrett (4), in the course of isolation of cathepsin D from chicken liver, obtained three homogeneous preparations differing in their electrophoretic mobility, but possessing the same molecular weight. In his opinion, these preparations are isoenzymes. Contrary to our isolation procedure, Barrett included autolysis in the first isolation step, and it is by this fact that the diversity of results can possibly be explained.

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