ALLOSTERIC PROPERTIES OF CATHEPSIN C IN NEUTRAL MEDIUM

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UDC 577.156.2.02

The effect of pH of the medium on the rate of hydrolysis of glycine-L-phenylalanine nitroanilide by cathepsin C from bovine spleen was investigated. At pH 7.25, the curve of initial velocity versus concentration of substrate for this enzyme is S-shaped, and Hill's index of cooperativeness has the value of 2. It is suggested that under normal physiological conditions cathepsin C functions at near-neutral pH values.

Cathepsin C (3.4.4.9) has a high molecular weight (220,000) and consists [1] of four subunits, each with a molecular weight of 55,000. The fact that cathepsin C has a quaternary structure suggests that this enzyme may have regulatory (allosteric) properties. To study this problem, glycyl-phenylalaninamide acetate has been used as substrate. However, the difficulties of working with low concentrations of this substrate have prevented unequivocal results from being obtained.

Gorter and Gruber [4] have recently shown that cathepsin C in fact possesses allosteric properties (at pH 5.0 and in a low concentration of Cl ions). These workers suggested the use of glycyl-L-phenylalanine-p-nitroanilide (GPNA) as the substrate. This substrate is free from the disadvantages of glycyl-phenylalaninamide and it enables the course of the reaction to be recorded spectrophotometrically.

In the investigation described below the rate of hydrolysis of GPNA* by cathepsin C was investigated in relation to pH of the medium.

EXPERIMENTAL METHOD AND RESULTS

Cathepsin C from bovine spleen, isolated as described previously [2] was used in the experiments. According to the results of analytical ultracentrifugation the enzyme contained 5-10% of low-molecular-weight impurities. Activity of the enzyme, calculated by the formula suggested by Gorter and Gruber [4], and using GPNA as the substrate, was 170 units/mg protein.

To investigate the relationship between the kinetic properties of cathepsin C and the pH of the medium, acetate buffer, pH 5.0, and phosphate buffer, pH 6.0, 6.7, and 7.25, of equal ionic strength (0.15) were used. The free incubation mixture consisted of 2 ml buffer of the appropriate pH, containing 125 mM sodium chloride and 150 mM cysteine hydrochloride, brought up to the pH of the buffer solution by the addition of alkali, and 0.4 ml of the enzyme (about 40 μg). After exposure of the preincubation mixture for 20 min, which was necessary to activate the enzyme, 0.6 ml of GPNA solution of the appropriate concentration and made up in the same buffer as the preincubation mixture was added. Preincubation and the reaction itself were carried out in a standard quartz cell of the SF-4 spectrophotometer in a thermostatically controlled receiver at 25°C. The reaction velocities were recorded from changes in optical density at 410 nm.

*GPNA hydrobromide was generously provided by Candidate of Chemical Sciences E. S. Chaman, to whom the authors are grateful.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 8, pp. 49-51, August, 1971. Original article submitted January 7, 1971.

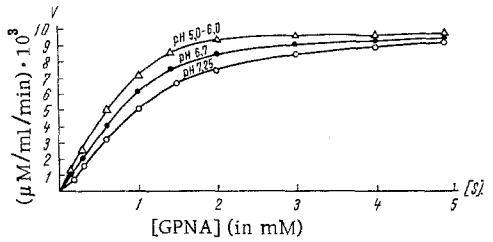

Fig. 1

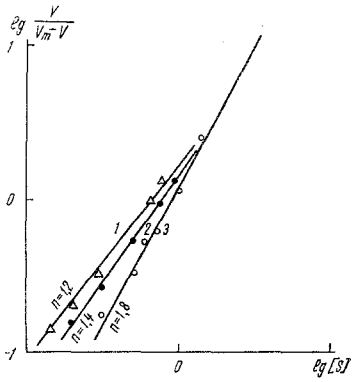

Fig. 2

Fig. 1. Velocity of hydrolysis of GPNA catalyzed by cathepsin C (ordinate) as a function of substrate concentration (abscissa) at pH 5.0-7.25.

Fig. 2. Hill's graph for determination of the index of cooperativeness: 1) pH 5.0-6.0; 2) pH 6.7; 3) pH 7.25.

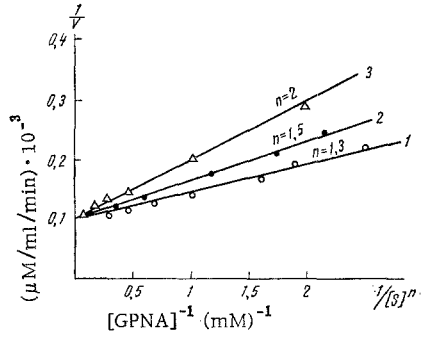

Fig. 3. 1/V versus $1/[S]^n$: 1) pH 5.0-6.0; 2) pH 6.7; 3) pH 7.25.

Distributive chromatography of the incubation mixture on Silufol in a chloroform–methanol–acetic acid (10:5:1 by volume) system demonstrated the absence of free glycine and phenylalanine. It was also shown that incubation of cathepsin C at neutral and weakly alkaline pH value does not inactivate the enzyme.

The curves of initial reaction velocity versus GPNA concentration obtained (Fig. 1) are varied in character. At pH 5.0-6.0 the curve is similar to the hyperbola described by the Michaelis–Menton equation. At pH 6.7 the curve deviates from hyperbolic, and at pH 7.25 it is S-shaped.

The index of cooperativeness was determined by Hill's classical equation. The results of analysis of the experimental data by Hill's method, shown in Fig. 2, demonstrate that this index changes its value from almost unity (at pH 5.0-6.) to a value of close to two (at pH 7.25).

GPNA is only sparingly soluble, it is difficult to obtain an accurate value for the maximum velocity. For this reason, the index of cooperativeness was also calculated by the method suggested by Kurganov et al. [3], which does not require preliminary determination of the maximum velocity. In this case values of 1.3, 1.5, and 2 were obtained for n at pH 5.0-6.0, 6.7, and 7.25 respectively. These values are in good agreement with those obtained by Hill's method.

Graphs of 1/V versus $1/[S]^n$ were plotted (Fig. 3) and values of the concentration of half-saturation ($[S]_{0.5}$) were calculated from them. These gave values of 0.6, 0.75, and 0.95 mM for pH values of 5.0-6.0, 6.7, and 7.25 respectively. These results indicate that a change of pH leads to a change in the affinity of substrate for enzyme, but does not affect the maximum velocity.

The presence of allosteric properties in cathepsin C at neutral pH, i.e., under physiological conditions, deserves note. The impression has been gained from the results of hydrolysis of several substrates (glycyl-phenylalaninamide, glycyl-tyrosinamide) at pH 5.0-6.0 that cathepsin C is an acid proteinase. However, this conclusion is not soundly based, for other works [5] have shown that certain naphthylamide derivatives of dipeptides are hydrolyzed by cathepsin C in the neutral and weakly alkaline pH zone. Cathepsin C can thus catalyze hydrolysis at neutral pH values also.

The possibility cannot be ruled out that under normal physiological conditions cathepsin C functions at near-neutral pH values.

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