INHIBITION OF CATHEPSIN E BY DIAZOACETYL-NORLEUCINE METHYL ESTER

H.KEILOVÁ and C.LAPRESLE
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, and Service de Chimie Microbienne, Institut Pasteur, Paris

Received 24 July 1970

1. Introduction

In a previous study [1] we described the inhibition of cathepsin D by diazoacetyl-norleucine methyl ester (DANME) * in the presence of copper ions. A similar inhibition has been observed before with pepsin [2, 3], in which an aspartic acid residue of the active site is blocked by the above reagent. This indicates a similar mechanism of catalytical action of the two enzymes.

Cathepsin E is an intracellular proteinase of rabbit bone marrow [4] with an optimum activity in the acidic pH-range, resembling cathepsin D in its specificity as assayed with the oxidized B-chain of insulin as substrate. The present study was undertaken to establish whether cathepsin E could be inhibited by the same reagent as cathepsin D and to find similar features of the inhibition mechanisms.

2. Materials and methods

Cathepsin E was a preparation purified according to [4] with the exception that the gel filtration step was performed on a column of Sephadex G-200 instead of G-75.

Enzyme assay: 1 ml of a 2 percent hemoglobin solution acidified to pH 2.5 (hydrochloric acid) and 0.2 ml of an 0.1 percent solution of cathepsin E were incubated 40 min at 40°. Under these conditions, the time dependence of the hydrolysis is linear. The reaction was stopped by the addition of 2 ml of 5 percent trichloroacetic acid, the precipitate was separated by filtration, and the absorbance of the filtrate was measured at 280 nm. The activity of cathepsin D samples was determined by the same assay at pH 3.5.

Inhibition assay: A mixture of the enzyme solution (8 mg in 7.2 ml of water), 4 M acetate buffer (0.4 ml, pH 5.0) and copper acetate (0.4 ml of a solution of varying concentration) was allowed to stand 15 min at room temperature. Methanolic solution of DANME was then added. Aliquots for activity assay were withdrawn at 10 min intervals.

3. Results and discussion

Bone marrow is the original source of cathepsin E. It has been shown that this proteinase occurs in large amounts in polymorphonuclear cells, in smaller quantities in macrophages, and only in trace amounts in lymphocytes, in contrast to cathepsin D, whose presence in all of these three cell types has been demonstrated [4].

The two proteinases are similar in their catalytic properties, but differ considerably in molecular weight and electrophoretic mobility. Whereas cathepsin D shows on agar gel electrophoresis at pH 8.2 a mobility of 1.7×10^{-5} cm^2 V^{-1} sec^{-1}, the corresponding value for cathepsin E is -7.2×10^{-5} cm^2 V^{-1} sec^{-1}. Both proteinases digest the haemo-

* *Abbreviation:*
DANME, diazoacetyl-norleucine methyl ester.

North-Holland Publishing Company – Amsterdam

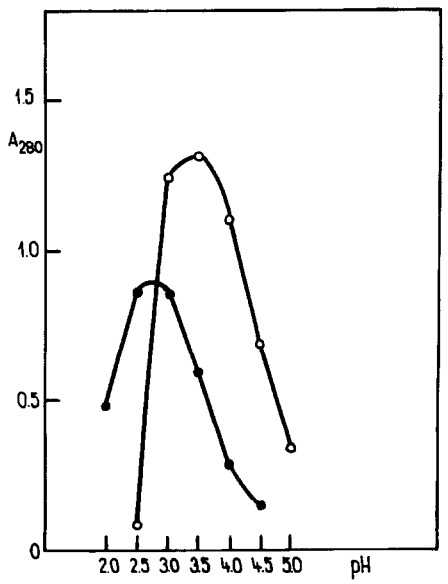

Fig. 1. pH-optimum of rabbit cathepsin D and E. The reaction mixture contained 2% of acid denaturated hemoglobin as substrate. o—o cathepsin D; •—• cathepsin E.

globin substrate at acidic pH-values. As demonstrated in fig. 1, using hemoglobin as substrate and under the experimental conditions described above, cathepsin D from rabbit spleen shows activity optimum at pH 3.5, whereas the optimum pH for the activity of cathepsin E lies at pH 2.5–2.8. These results are in good agreement with the data obtained before using serum albumin as substrate [4].

Neither cathepsin D nor cathepsin E are inhibited by diisopropylphosphofluoridate or sulfhydryl reagents. The possible presence of an active metallic complex has also been eliminated. On the other hand, the results of the present study show a specific inhibition by DANME in the presence of bivalent copper ions. Neither DANME (2.6 mg per mg of enzyme) nor copper acetate (mg per mg of enzyme) alone exert an inhibitory effect on cathepsin E. The presence of both these components is required for the inhibition. At constant concentration of the copper salt the inhibitory effect is dependent on the concentration of DANME (table 1A). An analogous dependence on the concentration of copper ions up to a certain saturation point can be demonstrated at a constant DANME concentration (table 1B).

Table 1
Inhibition of cathepsin E by DANME in the presence of copper acetate

(A) Incubation of the enzyme (1 mg) with copper acetate (100 μg) followed by addition of varying amounts of DANME;

DANME added (μg)	Residual activity
0	100
25	93
50	85
100	40
200	5–10
400	0

(B) Incubation of the enzyme (1 mg) with varying amounts of copper acetate followed by addition of DANME (200 μg)

Cu^{2+}-acetate added (μg)	Residual activity
0	100
10	75
50	13
100	5
300	0
900	0

The character of inhibition of cathepsin D from rabbit spleen is similar to that observed before with bovine spleen cathepsin D.

The result obtained in this study indicates that the mechanism of proteolytic activity of cathepsin E is similar to that of pepsin, rennin, *Penicillium janthinellum* protease, and cathepsin D [1, 5–7]. Despite the different location of these enzymes in the organism and of presumably also their different physiological function, cathepsins D and E are closely related both in specificity and mechanism of action. Similarities in the conformation of their active sites can therefore be expected.

References

[1] H.Keilová, FEBS Letters 6 (1970) 312.
[2] T.G.Rajagopalan, W.H.Stein and S.Moore, J. Biol. Chem. 241 (1966) 4295.
[3] R.S.Bayliss, J.R.Knowles and G.B.Wybrandt, Biochem. J. 113 (1969) 377.

[4] C.Lapresle and T.Webb, Biochem. J. 84 (1962) 455.
[5] B.Foltmann, Compt. Rend. Trav. Lab. Carlsberg 35 (1966) 143.
[6] J.Šodek and T.Hofmann, J. Biol. Chem. 243 (1968) 450.
[7] N.Camerman, T.Hofmann, S.Jones and S.C.Nyburg, J. Mol. Biol. 44 (1969) 569.