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EFFECT OF PEPSIN INHIBITOR FROM *ASCARIS LUMBRICOIDES* ON CATHEPSIN D AND E

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SUMMARY

We studied the effect of pepsin inhibitor, isolated from the body walls of the roundworm *Ascaris lumbricoides*, on the proteolytic activity of two acidic tissue proteinases, cathepsin D and E. Whereas the inhibitor decreased the rate of cleavage of hemoglobin by cathepsin E, it was without any effect on the proteolytic activity of cathepsin D. Likewise, it did not inhibit the proteolytic activity of rennin. We found that the inhibition is of the pseudo-irreversible type.

INTRODUCTION

Cathepsin D and E are tissue proteinases which, due to the character of their active site, fall into the group of the so-called acidic proteinases like pepsin, rennin, gastricsin and the proteinase from *Penicillium janthinellum*. Like all proteinases of this group, cathepsin D and E are inhibited irreversibly by diazoacetylnorleucine methyl ester[1,2]. This points to the essential role of one or several carboxyl groups in the mechanism of catalytic hydrolysis. Using this inhibitor we have been able to isolate from the active site of cathepsin D a peptide which is identical in its amino acid composition with an analogous peptide isolated from bovine and porcine pepsin[3–5]. Cathepsin D and E are very similar in their enzymatic characteristics, such as pH optimum of cleavage and specificity; they differ, however, in their molecular weights, electrophoretic mobilities and origin in biological material. Whereas cathepsin D has been isolated from various tissues, cathepsin E occurs predominantly in bone marrow, especially in polymorphonuclear leucocytes and in free macrophages from peritoneal exudates[6].

The present knowledge of naturally occurring inhibitors of acidic proteinases is relatively meagre. Umezawa and co-workers[7,8] have isolated from *Streptomyces* an inhibitor of molecular weight 708 which inhibited both pepsin and gastricsin. It has been shown later that this inhibitor, named pepstatin, also inhibits cathepsin D and E, and rennin[9]. In 1970, Peanasky and Abu-Erreish[10] reported the isolation of a pepsin inhibitor from the body walls of the roundworm *Ascaris lumbricoides*. The

inhibitor was enriched 7000 times, has a molecular weight of 8000 and its K_i value assayed with hemoglobin as substrate is $5.4 \cdot 10^{-10}$.

The similarity in the enzymatic characteristics of pepsin, cathepsin D and E led us to examine the enzymatic interactions of these tissue enzymes with the pepsin inhibitor from *Ascaris*.

MATERIAL AND METHODS

Pepsin (EC 3.4.4.1) was a commercial preparation of Worthington Biochemical Corp. Rennin (EC 3.4.4.3) was purchased from Koch-Light Ltd. Cathepsin D (EC 3.4.4.23) was isolated from bovine spleen in this laboratory[11] and from chicken liver by Dr Barrett, Strangeways Research Laboratories, Cambridge, U.K. Cathepsin E was prepared by Dr Lapresle, Institut Pasteur, Paris, France.

Pepsin inhibitor from *A. lumbricoides* was isolated in this laboratory by the method described by Peanasky and Abu-Erreish[10]. Unlike this author, who used SE-Cellex for the chromatography, we employed Sephadex-SE in our procedure.

Proteolytic activity was measured on hemoglobin substrate. The degree of hydrolysis of 2% acid-denatured hemoglobin was determined in terms of absorbance at 280 nm of the filtrate after precipitation by trichloroacetic acid. The pH of the incubation mixture was 2.1 for pepsin, 2.5 for cathepsin E, 3.5 for cathepsin D and rennin. The character of inhibition was determined according to the method of Morrison[12].

RESULTS AND DISCUSSION

Our investigation of the effect of the pepsin inhibitor from *Ascaris* on proteolytic activity has shown that there is an essential difference between cathepsin D and E. Whereas the inhibitor markedly decreases the rate of cleavage of hemoglobin by cathepsin E, it is without any effect on the proteolytic activity of cathepsin D isolated both from bovine spleen or chicken liver. To obtain a more complete picture we also examined rennin and found that the inhibitor is also without effect on its proteolytic activity. The results are given in Fig. 1. It would thus appear that the specificity of the pepsin inhibitor from *Ascaris* is relatively narrow, compared to pepstatin which inhibits all the acidic proteinases known so far.

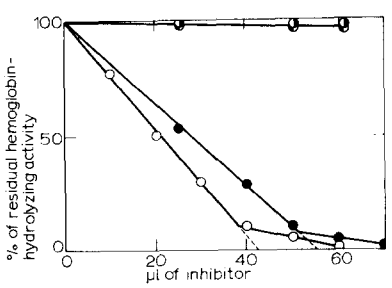
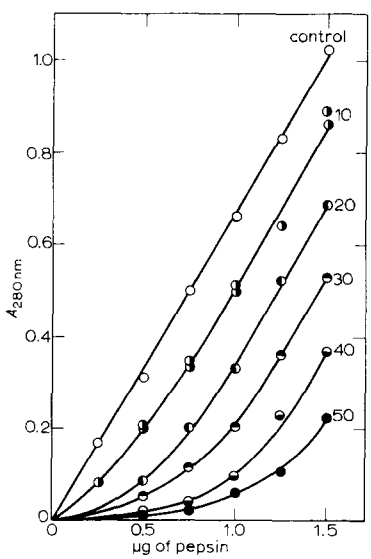


Fig. 1. Effect of pepsin inhibitor from *Ascaris* on hemoglobin-hydrolyzing activity of ○—○, pepsin (1 µg); ●—●, cathepsin E (300 µg); ◑—◑, cathepsin D (5 µg); and ◐—◐, rennin (200 µg). Temperature, 39 °C; time of incubation, 15 min (45 min for rennin). The absorbance of the inhibitor solution at 280 nm was $28 \cdot 10^{-3}$.

The differences in the behavior of cathepsin D and E toward the pepsin inhibitor from *Ascaris* indicate a different arrangement of those parts of the surface of the molecule which are necessary for the binding capacity of the inhibitor. From this viewpoint, it is interesting to compare the isoelectric points of the acidic proteinases used with their sensitivity to the inhibitor. It is, of course, clear that the reasons for the differences in their interaction with the inhibitor are most likely more complicated. Those proteinases which do not react with this inhibitor, such as rennin and cathepsin D, show isoelectric points at pH 4.6 (rennin) and at pH 5.2, 5.4 and 5.7 (the individual isoenzymes of cathepsin D). By contrast, the isoelectric point of pepsin is about 1; the isoelectric point of cathepsin E has not been determined so far, yet the character of this enzyme is strongly acidic judging by its electrophoretic mobility.

In an effort to establish whether the character of the inhibition by the inhibitor from *Ascaris* is reversible or irreversible, we determined the dependence of the rate of cleavage of hemoglobin on the concentration of cathepsin E in the presence of different concentrations of the inhibitor. We also carried out a similar experiment with pepsin. When the obtained results are expressed graphically, then the curves obtained all start at the origin. At higher concentrations of the enzyme they become parallel, and thus—according to the data recorded in literature—the type of the inhibition is pseudo-irreversible[12]. This plot for pepsin and cathepsin E is shown in Figs 2 and 3, respectively. The profile of the curves leads us to assume that the pepsin inhibitor from *Ascaris* can be characterized as a pseudo-irreversible or so-called tight-binding



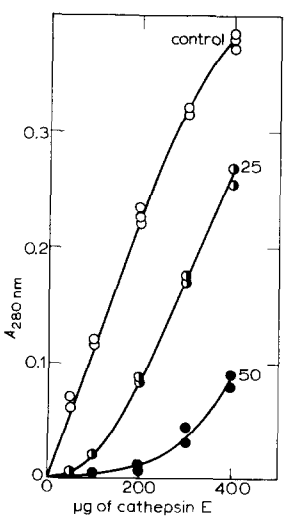


Fig. 2. Dependence of rate of cleavage of hemoglobin by various quantities of pepsin in presence of different concentrations of inhibitor. Temperature, 39 °C; time of incubation, 15 min. The absorbance of the inhibitor solution at 280 nm was $28 \cdot 10^{-3}$. The figures on the individual curves indicate the quantity of the inhibitor in μl.

Fig. 3. Dependence of rate of cleavage of hemoglobin by various quantities of cathepsin E in presence of different concentrations of inhibitor. Temperature, 39 °C; time of incubation, 15 min. The absorbance of the inhibitor solution at 280 nm was $28 \cdot 10^{-3}$. The figures on the individual curves indicate the quantity of the inhibitor in μl.

inhibitor. This type of inhibition also seems to be suggested by the K_i value reported for pepsin by Peanasky and Abu-Erreish[10].

In our opinion, the differences in the ability of cathepsin D and E to react with the pepsin inhibitor from *Ascaris* could be also employed for studies on the occurrence and role of cathepsin D and E in animal tissues.

ACKNOWLEDGEMENT

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REFERENCES

1 H. Keilová, *FEBS Lett.*, 6 (1970) 312.
2 H. Keilová and C. Lapresle, *FEBS Lett.*, 9 (1970) 348.
3 V. Tomášek and H. Keilová, unpublished results.
4 R. S. Bayliss, J. R. Knowles and G. B. Wybrandt, *Biochem. J.*, 113 (1969) 377.
5 P. A. Meitner, *Biochem. J.*, 124 (1971) 673.
6 C. Lapresle, in A. J. Barrett and J. T. Dingle, *Tissue Proteinases*, North-Holland Publishing Co., Amsterdam, 1971, p. 135.
7 H. Umezawa, T. Aoyagi, H. Morishima, M. Matsuzaki, M. Hamada and T. Takeuchi, *J. Antibiot. (Tokyo)*, 23 (1970) 259.
8 T. Aoyagi, S. Kunimoto, H. Morishima, T. Kakeuchi and H. Umezawa, *J. Antibiot. (Tokyo)*, 24 (1971) 687.
9 A. J. Barrett and J. T. Dingle, *Biochem .J.*, 127 (1972) 439.
10 R. J. Peanasky and Ghaleb M. Abu-Erreish, in H. Fritz and H. Tchesche, *Proc. Int. Res. Conf. on Proteinase Inhibitors, Munich, 1970*, Walter de Gruyter, Berlin, New York 1971, p. 289.
11 H. Keilová, in A. J. Barrett and J. T. Dingle, *Tissue Proteinases*, North-Holland Publishing Co., Amsterdam, 1971, p. 45.
12 J. F. Morrison, *Biochim. Biophys. Acta*, 185 (1969) 269.