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Elastinolytic activity of human cathepsin L

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The hydrolysis of a tritiated elastin substrate by the human cysteine proteinases cathepsins B and L has been studied. Cathepsin L was found to be at least 100-fold more active on this substrate than cathepsin B. The specific activity of cathepsin L at pH 5.5 for hydrolysis of elastin was about the same as that of pig pancreatic elastase at its optimum pH of 8.8.

INTRODUCTION

The essential characteristic of pulmonary emphysema is the irreversible destruction of the alveolar elastin. Although neutrophil elastase is though to be the enzyme principally responsible for this, other proteinases may also contribute. For example, lysosomal cysteine proteinases have been implicated in this disease. Cathepsin B-like activity has been found in bronchoalveolar lavage fluids (Orlowski *et al.*, 1981; Burnett & Stockley, 1985) and in sputum (Burnett *et al.*, 1983). Chapman & Stone (1984*a*) have reported that alveolar macrophages can digest elastin and that 60% of the macrophage elastinolytic activity could be attributed to an enzyme inhibited by Z-Phe-Ala-CHN_2 (Chapman & Stone, 1984*b*), which is a specific inhibitor of cysteine proteinases (Shaw, 1984).

Cathepsin B is the best characterized and most studied lysosomal cysteine proteinase. Cathepsin L is more rapidly inhibited by Z-Phe-Ala-CHN_2, however, and furthermore it degrades protein substrates such as azocasein and collagen at least an order of magnitude faster than cathepsin B (Barrett & Kirschke, 1981; Mason *et al.*, 1985). We have now examined the elastinolytic activities of cathepsins B and L in comparison with pig pancreatic elastase.

MATERIALS AND METHODS

Human cathepsin L was prepared as described by Mason *et al.* (1985) and human cathepsin B as described by Rich *et al.* (1986). Pig pancreatic elastase type III and pig trypsin type IX were obtained from Sigma Chemical Co., Poole, Dorset, U.K. ^{3}H-labelled elastin with a specific radioactivity of approx. 1.8 μCi/mg was prepared by reductive alkylation with boro[^{3}H]hydride (Gordon *et al.*, 1976). Molar concentrations of active sites of cathepsins L and B were determined by titration with E-64 as described by Barrett & Kirschke (1981); cathepsin L was assayed with Z-Phe-Arg-NHMec as described by Mason *et al.* (1985). The molar concentration of active trypsin was determined by titration with *p*-nitrophenyl *p'*-guanidinobenzoate as described by Chase & Shaw (1967). Elastase was titrated with Ac-Ala-Ala-Aala-$OPhNO_2$ as described by Powers & Gupton (1977). For assay of elastinolytic activity, ^{3}H-labelled elastin was washed three times in assay buffer and after the final wash suspended at a concentration of 5 mg/ml in assay buffer. The incubation mixture contained enzyme, 100 mM-sodium acetate buffer, pH 5.5, 1 mM-EDTA, 2 mM-dithiothreitol and 150 μg of ^{3}H-labelled elastin in a total volume of 0.5 ml. Samples were incubated at 37 °C with continuous mixing in a thermostatically controlled roller rack. Solubilization of radioactivity was measured by centrifuging the mixture and counting for radioactivity a 100 μl portion of the supernatant in a liquid-scintillation counter. For pig pancreatic trypsin and pig pancreatic elastase the incubation buffer was changed to 0.1 M-Tris/HCl buffer, at pH 8.0 and pH 8.8 respectively.

RESULTS

Human liver cathepsin L was found to hydrolyse elastin in a time-dependent manner (Fig. 1). The gradual decline in rate of solubilization could be accounted for partly by substrate depletion and partly by loss of enzyme activity. Only 70% of the initial activity of the enzyme in the incubation mixture remained after 6 h, as determined by assay with Z-Phe-Arg-NHMec.

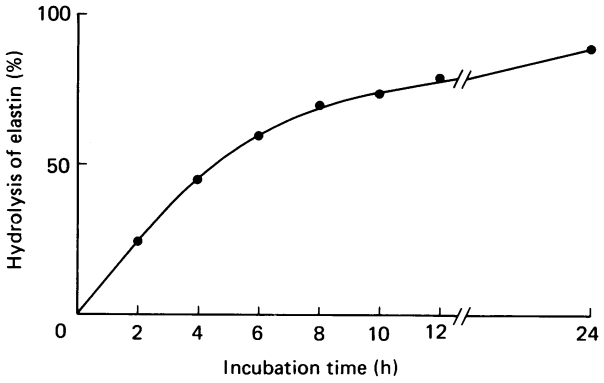

Fig. 1. Time-dependence of elastin hydrolysis by cathepsin L

Cathepsin L (160 nM) was incubated with ^{3}H-labelled elastin for 0, 2, 4, 6, 8, 10, 12 and 24 h at pH 5.5 as described in the Materials and methods section.

Abbreviations used: E-64, L-3-carboxy-*trans*-2,3-epoxypropionyl-leucylamido-(4-guanidino)butane; NHMec, 7-(4-methyl)coumarylamide; Z-, benzyloxycarbonyl; Aala, α-aza-alanine; -CHN_2, diazomethane; -$OPhNO_2$, *p*-nitrophenyl ester.

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Table 1. Comparison of the elastinolytic activities of cathepsin L and other proteinases

Cathepsins L and B were assayed at pH 5.5 as described in the Materials and methods section. Trypsin and elastase were assayed in 100 mM-Tris/HCl buffer, at pH 8.0 and 8.8 respectively. Incubation was for 24 h at 37 °C. Abbreviation: nd, not determined.

[Enzyme] (nM)	Amount of elastin hydrolysed (%)			
	Cathepsin L	Pancreatic elastase	Cathepsin B	Trypsin
6.7	2	2	nd	nd
13.5	6	11	nd	nd
20	8	15	nd	nd
33	17	83	< 1	< 1
67	62	100	< 1	< 1
166	100	95	< 1	< 1
333	71	94	3	3
1000	nd	nd	4	4
2000	nd	nd	8	5

The solubilization of elastin was shown to be dependent upon enzyme concentration (Table 1). At low concentrations of enzyme (< 20 nM) the elastinolytic activity of cathepsin L was found to be at least half that of pig pancreatic elastase.

At these concentrations trypsin and cathepsin B did not solubilize any elastin, 2 μM-enzyme being required to obtain any significant hydrolysis in 24 h. When more than 200 nM-cathepsin L was used, the amount of elastin solubilized was decreased; the reason for this is not known.

The pH optimum for hydrolysis of elastin by cathepsin L was in the range 4.5–5.5 (Fig. 2). There was no significant elastin hydrolysis at neutral pH.

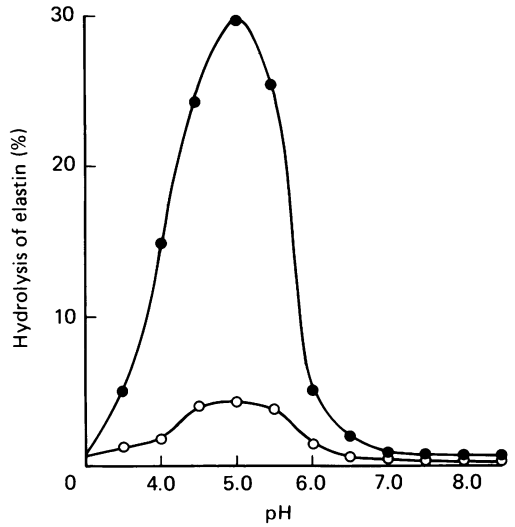

Fig. 2. pH-dependence of elastin degradation by cathepsin L

Cathepsin L (50 nM) was incubated with [^{3}H]elastin for 1 h (○) or 6 h (●) in 100 mM-buffer containing 2 mM-dithiothreitol as described in the Materials and methods section. Buffers used were sodium formate (pH 3.5–4.0), sodium acetate (pH 4.0–5.5) and sodium phosphate (pH 5.5–8.5).

DISCUSSION

Human cathepsin L has been shown to be a powerful elastinolytic enzyme at pH 5.5. Its activity against elastin is comparable with that of pig pancreatic elastase at its optimal pH, and hence is also similar to that of human leucocyte elastase (Starkey & Barrett, 1976). Furthermore, these enzymes are 100 times more active than elastase purified from mouse macrophages (Banda & Werb, 1981). Chapman & Stone (1984*b*) demonstrated that Z-Phe-Ala-CHN_2 inhibited elastinolytic activity of alveolar macrophages in culture. Z-Phe-Ala-CHN_2 is a much more effective inhibitor of cathepsin L than of other mammalian cysteine proteinases, including cathepsin B (Mason *et al.*, 1985), cathepsin H (Kirschke & Shaw, 1981) and calpain I (Parkes *et al.*, 1985). Furthermore, Z-Phe-Ala-CHN_2 is sufficiently hydrophobic to cross cell membranes directly (Grinde, 1982) and can be transported to lysosomes by pinocytosis (Shaw & Dean, 1984). Cathepsin L would therefore appear to be the enzyme responsible for Z-Phe-Ala-CHN_2-sensitive degradation of elastin by macrophages.

Cathepsin L, which is unstable above pH 6.0, showed no activity on elastin at higher pH values, and it is unlikely that cathepsin L could hydrolyse elastin in alveolar fluid. However, there must be some extracellular hydrolysis of elastin to provide particles suitable for phagocytosis. Although the local extracellular pH may be suitable for limited activity of cathepsin L (Etherington, 1980), the co-operative activity of a neutral-pH-stable elastase seems more likely. The present results, together with those of Chapman & Stone (1984*a*,*b*), suggest that cathepsin L may play a significant role in elastin degradation and/or remodelling in the lung. The elastinolytic activity of cathepsin L may be important in the pathogenic or normal solubilization of elastin in other tissues also.

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