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Proteolytic inactivation of human leukocyte elastase

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Summary. Human leukocyte elastase can be proteolytically inactivated by bovine pancreatic trypsin. Neither porcine pancreatic elastase nor bovine pancreatic chymotrypsin causes inactivation of leukocyte elastase, nor are trypsin, pancreatic elastase, or chymotrypsin themselves susceptible to proteolysis. The trypsin-catalyzed inactivation of leukocyte elastase can be slowed by inhibition of trypsin with benzamidine or by occupation of elastase's active site with elastatinal.
Key words. Elastase; inactivation, proteolytic.

Leukocyte elastase (EC 3.4.21.11) is thought to be the principle pathogenic agent involved in the development of pulmonary emphysema[1,2], a disease characterized by the progressive degradation of lung connective tissue, especially elastin[2,3]. Advances towards understanding the role played by this serine protease in emphysema have relied in large part on animal models. Emphysema can be mimicked in laboratory animals by intratracheal instillation of an elastolytic enzyme, typically porcine pancreatic elastase (PPE)[4]. Depending upon the dose, this single insult can result in a lesion that progressively worsens throughout the lifetime of the animal[4].

In attempts to potentiate the lesion by co-administration of elastase and another protease, we, and others, have found that bovine pancreatic trypsin (BPT) when administered with PPE to hamsters produces a significant enhancement of the resultant PPE-induced emphysema[5,6]. Surprisingly, we recently found that if trypsin is administered with human leukocyte elastase (HLE) the resultant lesion is significantly *diminished*[5]. Similarly, we observed that rates of elastinolysis were diminished by trypsin[7]. To explain the results regarding HLE we postulated two potential mechanisms[7]: a) the proteolytic inactivation of HLE by BPT or b) the generation of inhibitory peptides by BPT assisted elastinolysis. We now report results which demonstrate that HLE is in fact susceptible to proteolytic destruction by BPT.

Materials and methods. Human leukocyte elastase, prepared by the method of Viscarello et. al.[8], and porcine pancreatic elastase were obtained from Elastin Products, Pacific, MO. BPT, CT, and benzamidine were from Sigma Chemical Co. St. Louis, MO. Elastatinal was purchased from Vega Biochemicals, Tucson, Arizona.

Assays. HLE activity was measured with the chromophoric substrate MeOSuc-Ala-Ala-Pro-Val-pNA as previously described[8,9]. PPE[10], BPT[8], and CT[8], were also measured as previously described. Concentration of BPT active sites was determined with the titrant p-nitrophenyl-p'-guanidinobenzoate[11]. HLE concentration was determined from established kinetic constants for the hydrolysis of MeOSuc-Ala-Ala-Pro-Val-pNA[9].

Inactivation of HLE. The inactivation of HLE was followed by periodically assaying small aliquots (10–50 µl) of reaction solutions of HLE and BPT for residual activity against MeO-Suc-Ala-Ala-Pro-Val-pNA. Semi-log plots of residual HLE-activity vs time were linear for at least three half-lives.

Results. Susceptibility of HLE to proteolytic inactivation. Figure 1 contains results of an experiment in which HLE alone or in combination with equimolar concentrations (1µM) of either PPE, CT, of BPT was incubated and periodically assayed for HLE activity. It is apparent that while HLE is inactivated by BPT it is resistant to proteolysis by PPE or CT. The loss of

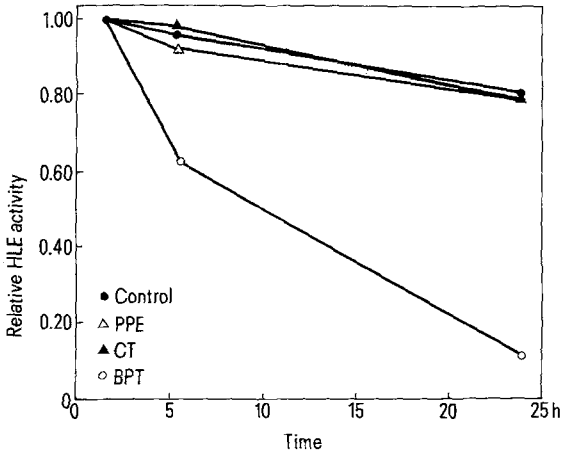

Figure 1. Effect of proteases on HLE activity. 1 µM HLE and an equimolar amount of another protease were incubated in 0.1 M Tris, 0.5 M NaCl, pH 8.0, 20 °C. Residual HLE activity is expressed relative to activity at time equal 1 min.

HLE activity in solutions containing no BPT is due to autolytic inactivation. Identical experiments were conducted to determine the susceptibility of each of these proteases to proteolytic inactivation. In no case was inactivation observed.

Kinetics of HLE inactivation by trypsin. Initial velocities were determined for the trypsin-catalyzed inactivation of HLE as a function of HLE concentration. Rates were linearly dependent on the concentration of HLE to concentrations as high as 32 μM. The absence of observable saturation kinetics indicates that the K_m for this reaction is well in excess of 32 μM and further suggests that at HLE concentrations around this value trypsinolysis should be first-order in HLE with $k_{obs} = (k_c/K_m) \times [BPT]$. As figure 2 demonstrates, HLE activity decreased exponentially with time and the observed first-order rate constants depended linearly on BPT concentration. From this figure an intercept equal to the autolysis rate constant and a slope equal to k_c/K_m can be extracted and were determined to be 2.5 sec^{-1} and 140 M^{-1} sec^{-1}, respectively.

Inhibition of trypsinolysis by benzamidine. Benzamidine, an inhibitor of trypsin[12], was found to inhibit the inactivation of HLE by BPT (fig. 3). The apparent K_i is 30 μM and is identical to the K_i determined for the inhibition of the BPT catalyzed hydrolysis of Bz-Phe-Val-Arg-pNA under these conditions (data not shown).

Effect of elastatinal. Elastatinal[13] is a competitive inhibitor of HLE with K_i equal to 40 μM (0.1 M Tris, 0.5 M NaCl, pH 8.0, 30 °C; data not shown). Figure 4 demonstrates that elastatinal protects HLE from autolytic inactivation and from proteolytic inactivation by BPT. The concentration of elastatinal used, 650 μM, has no effect on the BPT catalyzed hydrolysis of Bz-Phe-Val-Arg-pNA.

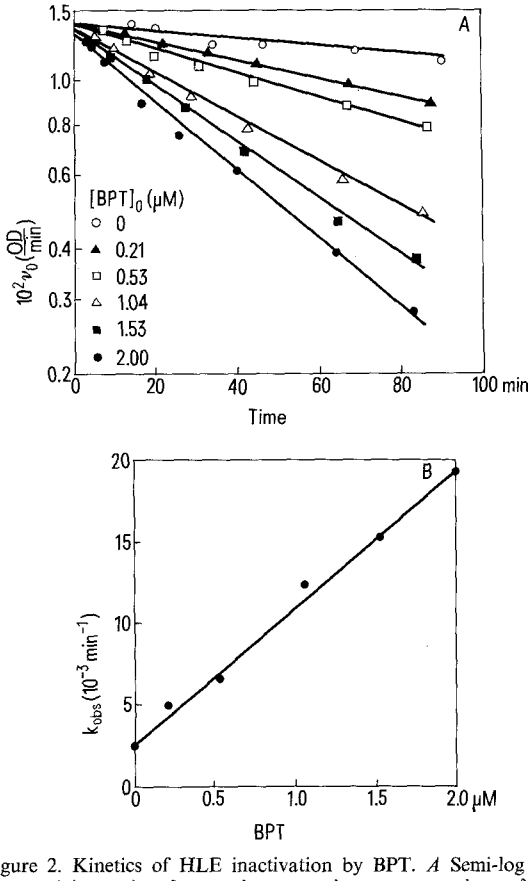

Figure 2. Kinetics of HLE inactivation by BPT. *A* Semi-log plot of HLE activity vs time for reactions at various concentrations of trypsin. $[HLE]_0$ = 15 μM. 0.1 M Tris, 0.5 M NaCl, pH 8.0, 30 °C. *B* Dependence of the observed first-order rate constant for HLE inactivation on trypsin concentration. k_{obs} was calculated from the slopes in the figure above.

Discussion. The results of these studies indicate that human leukocyte elastase is susceptible both to autolytic and to proteolytic inactivation. Although other reports of proteolytic inactivation of proteases can be found in the literature[14], this may not be a common phenomenon given that none of the other proteases examined here displayed such behavior.

Kinetic characterization of the hydrolysis of HLE by trypsin revealed a value of k_c/K_m equal to 140 M^{-1} sec^{-1}. Furthermore, the reaction is inhibitable by the competitive trypsin inhibitor benzamidine. Elastatinal, an HLE inhibitor, is also able to protect HLE from proteolytic destruction. This protection presumably results from some mechanism in which occupation of the active site by the inhibitor is able to stabilize a conformation of elastase that is resistant to proteolysis[15].

Physiological control mechanisms to prevent inappropriate tissue destruction may be many and varied. In addition to en-

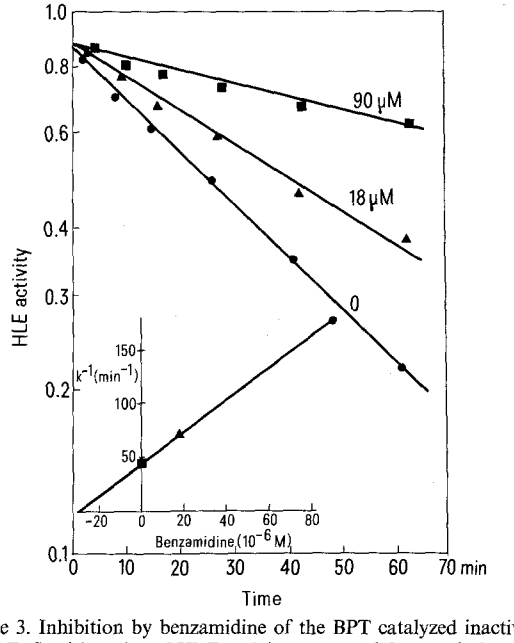

Figure 3. Inhibition by benzamidine of the BPT catalyzed inactivation of HLE. Semi-log plot of HLE activity, corrected for autolysis, vs time of reaction at various concentrations of benzamidine. $[BPT]_0$ = 1.0 μM, $[HLE]_0$ = 14 μM. 0.1 M Tris, 0.5 M NaCl, pH 8.0, 30 °C. Inset is a plot of reciprocal k_{obs} vs benzamidine concentration.

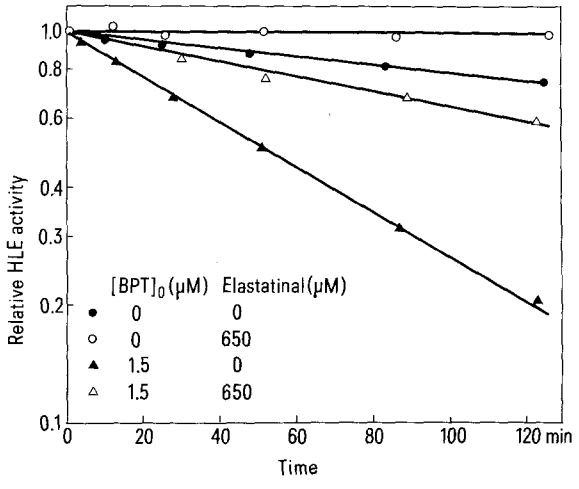

Figure 4. Effect of elastatinal on the trypsin catalyzed inactivation of HLE. Semi-log plot of HLE activity, relative to activity at time zero, vs time of reaction at various concentrations of BPT and elastatinal. $[HLE]_0$ = 15 μM: 0.1 M Tris, 0.5 M NaCl, pH 8.0, 30 °C.

dogenous protease inhibitors (e.g., alpha-1-protease inhibitor) our data suggest that the potential for direct proteolysis of HLE by an endogenous trypsin-like enzyme may offer an additional control mechanism for leukocyte elastase. Studies are presently underway exploring further biochemical aspects of the inactivation of HLE as well as accessing its physiological importance.

Abbreviations. HLE, human leukocyte elastase; PPE, porcine pancreatic elastase; BPT, bovine pancreatic trypsin β-trypsin; CT, bovine pancreatic α-chymotrypsin; MeOSuc-, methoxysuccinyl; pNA, p-nitroaniline.

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Bromocriptine-induced removal of endoplasmic membranes from prolactinoma cells[1]

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Summary. Adenomatous prolactin cells lose 39% of their cytoplasm volume within 7 days after the beginning of bromocriptine treatment. A simultaneous reduction of the rough-surfaced endoplasmic reticulum and the Golgi apparatus occurs. Their membranes are removed by rapid transport along the secretory pathway to the cell surface and to lysosomal destruction.
Key words. Pituitary neoplasms; prolactin; bromo-α-ergocriptine; endoplasmic reticulum; biological transport.

Prolactin (PRL) secreting pituitary adenomas (prolactinomas) exposed to bromocriptine for 4–6 weeks (daily dose 7.5 mg) demonstrate a cell shrinkage of 33–39% due to a reduction of the cytoplasm area with regression of the rough surfaced endoplasmic reticulum (RER) and the Golgi apparatus[2–4]. However, the mechanism of the reduction of the RER and of the Golgi apparatus has not yet been elucidated. Measurements of the RER and Golgi surface in the average tumor cell after a 1-week exposure to bromocriptine, i.e. during the phase of ongoing cell shrinkage, were made to obtain an insight into the processes that are active during the phase of endoplasmic membrane removal.

A new, injectable, long-acting form of bromocriptine ensures a therapeutic and sustained plasma drug level within hours after the injection[5]. Long-acting bromocriptine (50 mg) was injected 7 days before surgery in six patients bearing prolactinomas in order to achieve a shrinking and softening of the tumors and thereby facilitate their removal (short-term treatment) (Landolt, unpublished results). 10 biopsy specimens obtained from patients with prolactinomas were selected randomly from a large group of untreated patients; specimens from eight patients treated for 4–6 weeks with peroral bromocriptine (daily dose 7.5 mg) (chronic treatment) were used for comparison. The tissue was fixed in 2% S-collidine buffered osmium tetroxide and embedded in Epon. This fixation technique was preferred to the more commonly used glutaraldehyde-osmium fixation because the different types of cell membranes are better visible due to a 'washing-out' of cytoplasmic ground substance. The thin sections were contrasted with uranyl acetate and lead citrate[6]. Measurement of the average section area of the cell, nucleus, cytoplasm and the nucleolus was done on random electron micrographs of 100–140 cells (magnification $\times 3000$)[3]. The surface densities of the RER and Golgi membranes were measured on 30–40 random electron micrographs (magnification ×108,000) with the superimposed multipurpose test system (21 test lines) designed by Weibel et al.[7]. The total surface per average section of adenoma cell was calculated from the surface density and the cytoplasm area. The number of membrane-fixed ribosomes was determined by measuring the length of random profiles of RER membranes and counting the number of fixed ribosomes[8,9]. No attempt was made to correct for the section thickness[10] since this, as determined by interference color, and the final magnification of the electron micrographs were the same in all cases.

The results of the measurement of the size of the cells, cytoplasm, nuclei, nucleoli, surface of the RER and Golgi apparatus, the number of membrane-fixed ribosomes and ribosome density are shown in the table together with the results of the statistical comparison of the data with the H-test[11]. Median values are used to reduce the influence of outliers. The table shows that the major reduction of most parameters occurs within 1 week, with the exception of the ribosome density. The nuclear size does not decrease significantly.

We have calculated the half life of the cell volume, nucleolus volume, RER surface, Golgi surface and number and density of the membrane fixed ribosomes assuming that the shrinkage of these structures follows an exponential curve to the baseline of adenomas treated for 4–6 weeks (fig. 1). This baseline represents the new steady state since no further cell shrinkage was observed even if the bromocriptine treatment lasted one year[12]. The half life falls into the same range (2.2–3.7 days) for all parameters with the exception of the ribosome density (8.2 days) (table). No half life can be calculated for the cytoplasm volume and the nuclear volume.

In normal rat PRL cells examined in vitro, ergocryptine and bromocriptine treatment cause a rapid fall of PRL messenger RNA (mRNA) content and PRL release[13,14]. Both drugs block the DNA to mRNA translation of the PRL gene. Bromocrip-