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Kinetics of Proteolysis of Hog Gastric Mucin by Human Neutrophil Elastase and by *Pseudomonas aeruginosa* Elastase

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Human neutrophil and *Pseudomonas aeruginosa* elastases were compared for their ability to degrade hog gastric mucin, which was used as a model substrate. *P. aeruginosa* elastase was more active than neutrophil elastase, and 2 to 10 peptide bonds were hydrolyzed within 5 min. The results demonstrate that both elastases degrade mucins actively at concentrations comparable to physiological levels of neutrophil elastase, which raises the possibility that proteolysis of mucins may be one mechanism of damage during chronic infection and inflammation of the respiratory tract.

Lung secretions from chronically infected patients with bronchitis or cystic fibrosis contain numerous polymorphonuclear cells and large amounts of neutrophil elastase and cathepsin G (3, 8, 15). *Pseudomonas aeruginosa* elastase also appears to be present in the lungs of patients with cystic fibrosis (4), and the majority of patients with cystic fibrosis who are colonized with the organism have antibodies to the elastase (7). The degradation of tracheobronchial mucins with concomitant loss of their protective function may represent a mechanism in the pathology of chronic pulmonary disease. It has been observed that human tracheobroncheal mucins isolated from sputum samples from three patients with cystic fibrosis have lower molecular weights than mucins obtained from two normal controls (M. C. Rose, C. F. Brown, J. Z. Jacoby, W. S. Lynn, and B. Kaufman, Pediatr. Res., in press). It was hypothesized that proteolysis is responsible for the decrease in molecular weight. The purpose of the present study was to compare the rates of proteolysis of mucin by physiological concentrations of neutrophil elastase (3, 15) and identical concentrations of *P. aeruginosa* elastase. Hog gastric mucin was chosen as a model substrate because it is similar to human tracheal mucin in its carbohydrate and sulfate ester composition (1, 11). More importantly, the molar percentages of the amino acids that preferentially bind in the active sites of neutrophil elastase and *P. aeruginosa* elastase are similar in both mucins (1, 11).

Hog gastric mucin was purified by gel filtration chromatography on Bio-Gel A-50 agarose followed by batchwise adsorption on CM-52 resin, essentially as described previously (13, 14). Mucin concentrations were determined by the anthrone method (12), assuming a galactose content of 26% (wt/wt) (11). Hog gastric mucin was reductively methylated at pH 7.9 for 24 h at 20°C, essentially as described previously (10). The concentration of amino groups was determined with fluorescamine (10), and approximately 90% of the lysyl residues of methylated mucin was derivatized.

Mucin degradation was studied at 37°C in 1.0 ml of 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid–100 mM NaCl (pH 7.20). The experiments with equal molar amounts of elastase contained 0.96 mg of reductively methylated mucin and 38 μg of neutrophil elastase or 50 μg of *P. aeruginosa* elastase. The reactions were started by the addition of elastase. Samples (25 to 100 μl) were taken at 0, 5, 30, and 45 min and at 2, 4, and 6 h, and the concentration of amino groups was determined by fluorescamine analysis. Controls lacking either elastase or mucin were incubated and assayed in parallel, and the values from the controls were subtracted from the values for the test solutions.

The shape of the plots of amino group concentration produced by proteolysis versus time was hyperbolic over a 6-h incubation period. It did not vary substantially over an eightfold range of mucin concentrations (0.40 to 3.22 mg of mucin per ml; mucin/proteinase ratios of 100:1.9 to 100:27 [wt/wt]), and 2 mM $CaCl_2$ had little effect on the activity of either elastase (data not shown). The extent of mucin proteolysis by equal molar amounts of the two elastases was determined after we demonstrated that our neutrophil elastase preparation was greater than 95% active. This determination was made by comparing the specific activity of our preparation to that reported previously for a preparation of titrated neutrophil elastase (8). *P. aeruginosa* elastase hydrolyzed the mucin faster than neutrophil elastase (Table 1).

In this study, proteolysis of mucin was followed by direct measurement of the concentration of newly formed amino groups. Fluorescamine has been used previously for quantifying the rates of proteolysis of protein substrates (17). Reductively methylated mucin was used as the substrate to reduce the values for the mucin blanks in fluorescamine analysis. Since neither of the elastases catalyzed the hydrolysis of peptide bonds adjacent to lysyl residues, it is quite unlikely that methylation alters susceptibility to these proteases. The methylation of lysyl residues has been shown to have minimal effect on the conformation or activity of proteins, except when lysyl residues are required for activity (6).

The concentration of elastases that we chose for our study, 26 to 100 μg/ml, was in the same range as that found for neutrophil elastase in sputum samples from infected bronchitic patients (15) and from cystic fibrosis patients (3). Our studies (2) have found approximately equal levels of noninhibited serine elastase and metalloelastase activities in sputum samples from patients with cystic fibrosis. There-

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TABLE 1. Determination of extent of proteolysis of hog gastric mucin by equal molar amounts of *P. aeruginosa* and neutrophil elastases

Time (min)	Concn (μM) of amino groups[a]:		P^b
	P. aeruginosa elastase	Neutrophil elastase	
5	5.6 ± 3.1	1.2 ± 1.7	<0.02
30	17.5 ± 3.3	3.3 ± 1.9	<0.01
45	20.0 ± 3.3	4.3 ± 2.5	<0.01

[a] Mean ± confidence interval for 20 or 21 determinations. Paired *t* test indicated that proteolysis of mucin was significantly different ($P < 0.05$) from autoproteolysis and autohydrolysis of mucin.
[b] Determined by unpaired *t* test.

fore, we elected to study the effects of equivalent concentrations of neutrophil and bacterial elastases on rates of mucin proteolysis. It should be noted that other studies (3, 5, 16) have found that all or the majority of proteolytic activity in lung secretions from such patients is derived from granulocytes. Further work with enzyme-specific irreversible inhibitors is being done to resolve this discrepancy.

It is difficult to account for the difference in rates of proteolysis of hog gastric mucin by the two elastases, because both enzymes are capable of rapidly hydrolyzing artificial substrates and their specificities are similar (9, 18). The molar percentages of the relevant amino acids in hog gastric mucin (11) are as follows: Ala (4.8), Ile (3.6), Leu (3.6), Phe (1.7), Tyr (1.9), and Val (5.7). Perhaps the difference in rates of proteolysis is a function of the concentration of these residues in the nonglycosylated portions of the mucin.

Our results indicate that, on a molar basis, mucin is at least 50-fold less sensitive to the two elastases than is casein (data not shown). However, both elastases were used in these experiments at concentrations comparable to physiological levels of neutrophil elastase, and it is clear that mucin is degraded at a reasonably rapid rate. After 5 min, 2 to 10 peptide bonds were cleaved by the elastases (calculation based on a mucin molecular weight of 2×10^6) (11). This level of in vitro mucin degradation suggests that substantial degradation of mucins may occur in vivo. The fact that tracheobronchial mucins from patients with cystic fibrosis have lower molecular weights than mucins from controls (Rose et al., in press) is consistent with proteolytic degradation. Cleavage of even one peptide bond is likely to substantially decrease the molecular weight of the mucin, with concomitant alterations in mucin physical properties.

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LITERATURE CITED

1. **Boat, T. F., P.-W. Cheng, and R. E. Wood.** 1977. Tracheobronchial mucus secretion *in vivo* and *in vitro* by epithelial tissues from cystic fibrosis and control subjects. Mod. Probl. Paediatr. **19**:141–152.
2. **Bruce, M. C., L. Poncz, J. D. Klinger, R. C. Stern, J. F. Tomashefski, Jr., and D. G. Dearborn.** 1985. Biochemical and pathological evidence for proteolytic destruction of lung connective tissue in cystic fibrosis. Am. Rev. Respir. Dis. **132**:529–535.
3. **Goldstein, W., and G. Doring.** 1986. Lysosomal enzymes from polymorphonuclear leukocytes and proteinase inhibitors in patients with cystic fibrosis. Am. Rev. Respir. Dis. **134**:49–56.
4. **Hoiby, N.** 1982. Microbiology of lung infection in cystic fibrosis patients. Acta Paediatr. Scand. Suppl. **301**:33–54.
5. **Jackson, A. H., S. L. Hill, S. C. Afford, and R. A. Stockley.** 1984. Sputum sol-phase proteins and elastase activity in patients with cystic fibrosis. Eur. J. Respir. Dis. **65**:114–124.
6. **Jentoft, N., and D. G. Dearborn.** 1983. Protein labeling by reductive alkylation. Methods Enzymol. **91**:570–579.
7. **Klinger, J. D., D. C. Straus, C. B. Hilton, and J. A. Bass.** 1978. Antibodies to proteases and exotoxin A of *Pseudomonas aeruginosa* in patients with cystic fibrosis. J. Infect. Dis. **138**:49–58.
8. **Martodam, R. R., R. J. Baugh, D. Y. Twumasi, and I. E. Liener.** 1979. A rapid procedure for the large scale purification of elastase and cathepsin G from human sputum. Prep. Biochem. **9**:15–31.
9. **Morihara, K., and H. Tsuzuki.** 1975. *Pseudomonas aeruginosa* elastase: affinity chromatography and some properties as a metallo-neutral proteinase. Agric. Biol. Chem. **39**:1123–1128.
10. **Poncz, L., and D. G. Dearborn.** 1983. The resistance to tryptic hydrolysis of peptide bonds adjacent to N,N-dimethyllysyl residues. J. Biol. Chem. **258**:1844–1850.
11. **Scawen, M., and A. Allen.** 1977. The action of proteolytic enzymes on the glycoproteins from pig gastric mucus. Biochem. J. **163**:363–368.
12. **Shields, R., and W. Burnett.** 1960. Determination of protein-bound carbohydrate in serum by a modified anthrone method. Anal. Chem. **32**:885–886.
13. **Shogren, R., A. M. Jamieson, J. Blackwell, and N. Jentoft.** 1986. Conformation of mucous glycoproteins in aqueous solvents. Biopolymers **25**:1505–1517.
14. **Snary, D., and A. Allen.** 1972. Studies on gastric mucoproteins: the production of radioactive mucoproteins by pig gastric mucosal scrapings *in vitro*. Biochem. J. **127**:577–587.
15. **Stockley, R. A., and D. Burnett.** 1980. Serum derived protease inhibitors and leukocyte elastase in sputum and the effect of infection. Bull. Eur. Physiopathol. Respir. **16**(Suppl.):261–271.
16. **Suter, S., U. B. Schaad, L. Roux, U. E. Nydegger, and F. A. Waldvogel.** 1984. Granulocyte neutral proteases and pseudomonas elastase as possible causes of airway damage in patients with cystic fibrosis. J. Infect. Dis. **149**:523–531.
17. **Wunderwald, P.** 1984. Proteinases (proteins as substrates), p. 258–270. *In* J. Bergmeyer and M. Grassl (ed.), Methods of enzymatic analysis, vol. 5. Verlag Chemie, Deerfield Beach, Fla.
18. **Yasutake, A., and J. C. Powers.** 1981. Reactivity of human leukocyte elastase and porcine pancreatic elastase toward peptide 4-nitroanilides containing model desmosine residues. Evidence that human leukocyte elastase is selective for cross-linked regions of elastin. Biochemistry **20**:3675–3679.