Microbial Elastases. A Comparative Study.* (27379)

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The insoluble fibrous protein constituents of connective tissue, collagen and elastin, resist degradation by common proteolytic enzymes such as trypsin or chymotrypsin. The only enzymes capable of degrading native collagen are specific clostridial collagenases (1) and these are without effect on elastin. Early reports in the literature indicated enzymic digestion of ligamentum nuchae elastin(1,2) but no specific elastase was known until 1949 when Balo and Banga(3) discovered and isolated elastase from mammalian pancreas. Since the level of this enzyme was found to change in atherosclerosis and old age(1) it was thought possible that elastase might have therapeutic applications. In addition a specific enzyme is a valuable tool in elucidation of the structure of its substrate, elastin, which is an important constituent of the arterial wall. Large scale production of pancreatic elastase is, however, difficult and expensive. No microbial elastase had been isolated until 1959 when Mandl and Cohen (2) obtained a specific elastase from a Flavobacterium species. This enzyme was found to differ from the pancreatic elastase in some of its properties; in particular its pH optimum was much lower, nearer the physiological, and it resisted inhibition by 1% NaCl and animal sera in 1:100 dilutions(2). More recently specific elastolytic activity has been observed in proteolytic enzyme preparations from other microbial sources(1,2) and it appears that microbial elastases are relatively widespread. The present study was undertaken to compare some of these elastolytic enzymes with respect to their pH optima and the effect of potential inhibitors. Such a survey of various elastases should indicate whether the microbial enzymes resemble each other and the pancreatic elastase and what differences must be considered if microbial elastases are to be applied for either clinical or structural studies.

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Materials and methods. Three Pseudomonadaceae, 3 Bacillaceae and 4 Actinomycetaceae enzyme preparations were compared with crystalline pancreatic elastase (Worthington Corp., Freehold, N.J.) and a crude, ammonium sulfate precipitated, Flavobacterium elastase(2). Of these preparations 3 were isolated in our laboratory (Ps-1, Ps-2 and B-1), 2 were obtained through the courtesy of Dr. R. J. Heckly (Ps-3) and Dr. H. Cohen (B-2) respectively and 5 are commercial preparations (B-3, A-1, A-2, A-3, A-4). Of the latter B-3 and A-1 were kindly supplied to us by Drs. A. L. Everett and T. C. Cordon who also first described the elastolytic activity of these enzymes(4,5). Table I lists the enzymes involved. It may be added that several additional strains of *Ps. aeruginosa, B. subtilis* and Actinomyces as well as other Flavobacterium species were tested and did not show any elastase activity.

Assays were performed by a modification of the orcein-elastin test(10). Twenty mg orcein-elastin (Sigma Corporation, St. Louis, Mo.) were incubated at 37° for 3 hours with 1 mg of the microbial preparations in a total volume of 3 ml. The undigested substrate was centrifuged off and the supernatant color read in a Bausch and Lomb Spectronic 20 colorimeter at 590 mμ. Optical density was compared with a straight line calibration curve obtained with different amounts of completely digested orcein-elastin; the readings were converted to per cent hydrolysis. Phosphate, Tris and carbonate buffers, 0.03 M, were used for pH determinations, Tris buffer, 0.03 M, pH 8.5 for the inhibitor studies. One-tenth mg enzyme per 3 ml was used for the comparative pancreatic elastase studies and additional inhibition tests at pH 7.4 were made with the Flavobacterium enzyme. NaCl to a final concentration of 1% and normal human serum to final concentrations of 1:10, 1:50 and 1:100 from a single batch obtained through the Francis Delafield

TABLE I. Microbial Enzyme Preparations Used.

Organism	Source
Pseudomonadaceae	
Ps-1 *Ps. aeruginosa*	Periodontal pocket(6)
Ps-2 *Ps. aeruginosa*	Collagen digest contaminant
Ps-3 *Ps. pseudomallei*	Heckly and Nigg(7)
Bacillaceae	
B-1 *B. subtilis*	Exposed elastin - agar plate
B-2 *B. subtilis*	H. Cohen, Princeton Laboratories, N. J.
B-3 *B. subtilis*	Miles, Clifton, N. J. HT - proteolytic(4)
Actinomycetaceae	
A-1 *Streptomyces fradiae*	Merck, Sharp & Dohme, Rahway, N. J. Keratinase(8,5)
A-2 *Streptomyces griseus*	California Corp. for Biochemical Research, Los Angeles, Cal. Pronase B(9)
A-3 *Actinomyces species*	Lederle Laboratories, Pearl River, N. Y. Proteinase D
A-4 *Actinomyces species*	Agricultural Biological Corp., Lynbrook, L.I., N. Y. Fungal elastase

Hospital blood bank were added in the inhibition tests.

Results. Fig. 1 shows the pH activity curves obtained with the enzymes listed in Table I. All 10 microbial elastases have pH optima in the alkaline range. The mold enzymes have broad maxima but in all cases their activities between pH values of 8.5 and 9.5 are higher than at the Flavobacterium elastase optimum of pH 7.4. Most but not all of the activities are higher in Tris buffer than in phosphate buffer of the same pH. Table II gives the per cent residual inhibition of elastolytic activity of the same microbial enzyme preparations in presence of NaCl (1%) and human serum (1:50). At these concentrations pancreatic elastase was completely inhibited whereas the Flavobacterium elastase was not affected. NaCl was found to inhibit the elastolytic activity of all microbial enzymes tested in varying degree comparable to that observed with the pancreatic enzyme; the effect of serum in 1:50 dilution, on the other hand, was much less on the microbial elastases than on the pancreatic enzyme. In higher concentrations (1:10) this serum almost completely inhibited all the microbial elastases including the Flavobacterium enzyme.

TABLE II. Effect of Salt and Human Serum on Elastolytic Activity of Microbial Enzymes.

Enzyme	% inhibition 1% NaCl	1:50 Serum
Ps-1	46	0
Ps-2	59	19
Ps-3	92	64
B-1	81	13
B-2	47	39
B-3	73	11
A-1	19	28
A-2	43	7
A-3	65	14
A-4	52	14

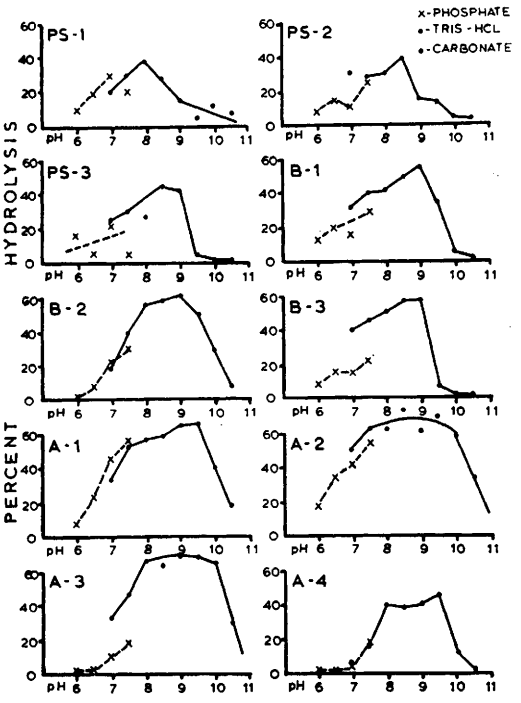

FIG. 1. pH activity curves of microbial elastases.

Discussion. The results obtained indicate that the Pseudomonas, *B. subtilis* and Actinomycetaceae elastases tested resemble pancreatic elastase in the pH optima and in their susceptibility to the inhibitory effect of NaCl. Serum inhibition of these microbial enzymes

is less pronounced than that of the pancreatic elastase and comparable to that observed with Flavobacterium elastase.

Various pH optima have been reported for pancreatic elastase, depending on conditions used(1); the best value is probably that of Lamy *et al.*(11) who found optimum activity between 9.2-9.4. With orcein-elastin assays in Tris buffer(10) the optimum pH is 8.8, while Flavobacterium elastase with this same test showed optimum activity at 7.4(2). Sbarra and Gilfillan(12) in their report of the elastase activity of Actinomycetes species stated that the optimum pH of their enzymes was similar to that of the pancreatic elastase and Comte *et al.*(13) found elastolytic activity of a *B. subtilis-mesentericus* protease highest around pH 9.8. The pH optima of the preparations tested by us also resemble that of the pancreatic enzyme.

NaCl has been shown to inhibit pancreatic elastase(14,15,16). Physiological saline inhibited 50-75%(15,16) and other salts, *i.e.*, KCl(14,15), $(NH_4)_2SO_4$(14) $MgCl_2$, $CaCl_2$, Na_2SO_4, NaI(15) showed a similar effect. The inhibitory action is believed to be due to increase in ionic strength rather than a specific ion. Elastolytic activity of a *B. subtilis-mesentericus* enzyme was equally inhibited by neutral salts(13). Again the 10 microbial enzymes are more similar to the pancreatic than the Flavobacterium elastase. This is not true when the effect of human serum is considered. Tolnay and Bagdy(15) reported 50-90% inhibition with 1:100 dilutions of human, cattle, rabbit and guinea pig serum. Under the conditions used in this study 1:50 dilutions of human serum caused 100% inhibition of the pancreatic elastase activity. Inhibition of the microbial enzymes by this serum was much less pronounced. Graham(17) found that inhibition of pancreatic elastase by serum can be completely reversed by trypsin. We have been able to confirm this reversal of inhibition of pancreatic elastase and have observed similar reversal of the inhibition of Flavobacterium and other microbial elastases by more concentrated sera (1:10 dilutions).

Summary. The effects of pH and of 1% NaCl and human serum on the elastolytic activity of 10 microbial enzyme preparations were determined and compared with those previously reported for pancreatic elastase and Flavobacterium elastase. The pH optima and the NaCl inhibition of the microbial enzymes showed similarity with pancreatic elastase rather than Flavobacterium elastase, but serum inhibition was less pronounced.

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