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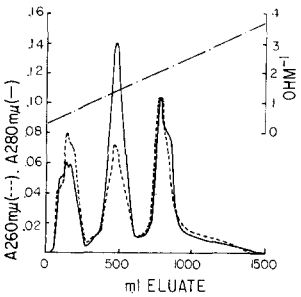
Two distinct elastases from different strains of *Pseudomonas aeruginosa*

A relationship between proteases and genesis of the hemorrhagic lesions associated with *Pseudomonas aeruginosa* infections may be inferred from literature data[1]. MULL AND CALLAHAN[2] reported loss of arterial elastic laminae in experimentally infected animals, and JOHNSON *et al.*[3] prepared a highly purified elastolytic protease from *P. aeruginosa* that was toxic for mice and induced dermonecrosis in rabbits. MORIHARA *et al.*[4] found that elastases produced by one strain of *P. aeruginosa* grown on meat extract and on semisynthetic media had identical chromatographic and enzymic properties. The anion-exchange chromatographic elution patterns of two highly purified elastases produced by two different laboratories from different strains suggest similarity of the enzymes[3,4].

During the present investigation of elastase-producing strains of *P. aeruginosa*, two physicochemically distinct elastases were found, isolated, and compared.

Cultures were grown on solid trypticase soy agar medium (Difco Laboratories, Detroit) at 30° for 18–24 h. Surface growth cell harvests were suspended in 0.15 M KCl and shaken with glass beads for 10 min. Cellular debris was removed by centrifugation. Crude elastases were precipitated from the cell-free extracts on addition of 4 vol. of cold ethanol. After refrigeration overnight, the precipitates were recovered by high-speed centrifugation and dissolved in volumes of deionized water equal to 1/10 the original extract volumes. These materials were dialyzed against 0.002 M potassium phosphate buffer, pH 7.5, containing 1% *n*-butanol and chromatographed on DEAE-cellulose columns equilibrated with the same buffer. Columns were developed at 5° with linear salt gradients generated from the equilibration buffer and equal volumes of 0.03 M potassium phosphate buffer, pH 7.5, containing 1% *n*-butanol. Eluates were monitored by absorbance measurements at 260 and 280 mμ. Salt gradients were monitored by measuring the conductivity of eluates. Proteolytic activities were detected qualitatively by the ability of eluates to digest the gel coatings of photographic films. Elastolytic activities were confirmed by digestion of elastin-agar "microcolumns" containing a 1% suspension of elastin (Mann Research Laboratories, New York) in 1.5% agar buffered at pH 7.6. Elastase-containing fractions were pooled and concentrated by dialysis against polyethylene glycol (mol. wt. 6000; J. T. Baker Chemical Co., Phillipsburg, N.J.). Purified elastases were quick-frozen in dry ice–acetone and stored at −20° with no loss of activity.

Protein concentrations of purified elastases were determined employing the optical extinction coefficient of 14.5 for a 1% solution at 280 mμ[4]. Electrophoretic properties were examined by disc electrophoresis in 7.5% polyacrylamide gels at pH 8.6 (ref. 5). Immunochemical reactivities were assessed in agar diffusion plates employing rabbit antisera to purified elastases. Proteolytic specific activities of the elastases were determined from the initial velocities of casein digestion, since casein is particularly sensitive to the enzymes with no lag phase apparent. An elastase caseinolytic unit was defined as the amount of enzyme that releases 0.10 μmole of perchloric acid-soluble tyrosine equivalents per ml of solution per min at 37° from a 0.7% solution of α-casein (Worthington Biochemical Corp., Freehold, N.J.) in 0.06 M Tris buffer at pH 7.5 containing 0.09 M NaCl. Elastases were chromatographed on



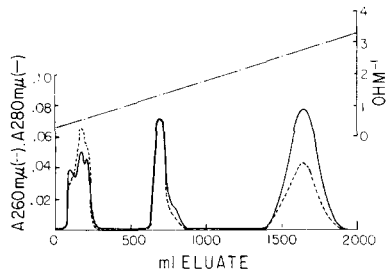
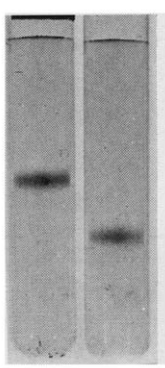


Fig. 1. Elution profile of elastase from the strain NYS 64–332 chromatographed on DEAE-cellulose. Elastolytic activity was found only in Peak 2.

Fig. 2. Elution profile of elastase from strain NYS 66-319 chromatographed on DEAE-cellulose. Elastolytic activity was found only in Peak 3.

Sephadex G-100 in 0.15 M NaCl and 0.05 M sodium phosphate, pH 7.5, for possible indications of associated states of the enzyme.

Distinct elution profiles were observed for elastases produced by *P. aeruginosa* strains NYS 64-332 and NYS 66-319 when chromatographed on DEAE-cellulose. These elastases differed by their respective elutions at conductivities of 1.35 Ω^{-1} (Peak 2 in Fig. 1) and 2.15 Ω^{-1} (Peak 3 in Fig. 2). This apparent increased net negative electrostatic charge of the elastase from strain NYS 66-319 was confirmed by its increased electrophoretic mobility at pH 8.6 as compared with that of the elastase from strain NYS 64-332 (Fig. 3). The sharpness of the single bands obtained for each elastase suggested their electrophoretic homogeneity (Fig. 3). Since the elution profile of the elastase from strain NYS 64-332 (Fig. 1) was similar to those previously published[3,4], the elastase produced by strain NYS 66-319 is believed to be a distinct type. Immunochemical identity of both elastases was indicated by absence of "spurring" on common precipitin arches (Fig. 4). Similarity of the proteolytic activities of both



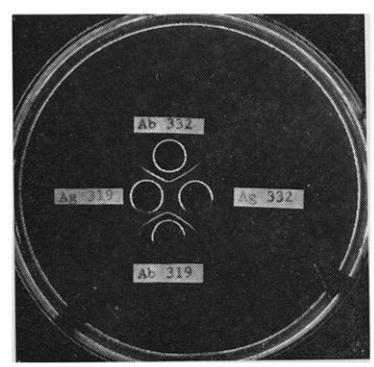


Fig. 3. Disc electrophoresis of purified elastases from strain NYS 64-332 (left) and strain NYS 66-319 (right). Samples (approx. 400 μg of protein) were electrophoresed in 7.5% polyacrylamide at pH 8.6. 5 mA per sample were applied for 2 h.

Fig. 4. Agar diffusion plate of purified elastases from strain NYS 64-332 (Ag 332) and strain NYS 66-319 (Ag 319) and rabbit antisera to these respective antigens (Ab 332 and Ab 319).

elastases was suggested by their specific activities of 1630 elastase caseinolytic units/mg of NYS 64-332 elastase (S.D. ± 215 for 10 determinations) and 1550 elastase caseinolytic units/mg of NYS 66-319 elastase (mean of 3 determinations). The respective distribution coefficients of 0.59 and 0.61 for NYS 64-332 and NYS 66-319 elastases on gel filtration in Sephadex G-100 suggest that the observed differences on ion-exchange chromatography and polyacrylamide electrophoresis are not attributable to association reactions. Thus, both elastases possess approximately the same molecular weights and proteolytic properties and are immunochemically indistinguishable. They differ in electrostatic charges as indicated by disc electrophoresis and ion-exchange chromatography. These differences in net charge do not produce immunologic distinctions.

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Esters of phenols as substrates for pancreatic lipase

Esters of phenol[1], *p*-nitrophenol, *β*-naphthol[2], 4-methyl umbelliferone[3,4], and fluorescein[5] have been used as substrates for "lipase" in colorimetric or fluorimetric analytical methods. It has also been reported that lipases, and in particular pancreatic lipase, show maximal activity against the medium chain length esters of these phenols[2–4]. If we agree to give the name lipase (EC 3.1.1.3) only to those enzymes which attack emulsified long-chain triglycerides, typically triolein or olive oil[6], we may doubt whether phenol esters will be appropriate substitutes for the natural substrate of such enzymes. I found that *p*-nitrophenyl oleate and *β*-naphthyl oleate are only slowly hydrolyzed by crude porcine pancreatic lipase and that phenyl oleate is not at all hydrolyzed[7]. BARROWMAN AND BORGSTRÖM[8] have recently conducted semi-quantitative assays on a number of phenol esters as substrates for the lipase and for an esterase of rat pancreatic juice. They found that triolein, *β*-naphthyl laurate and oleate, and *p*-nitrophenyl laurate were rapidly hydrolyzed by the lipase, whereas 4-