EVIDENCE THAT THE ACTIVITY OF ELASTASE IS NOT DEPENDENT ON THE IONIZATION OF ITS N-TERMINAL AMINO GROUP

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Summary

N-benzoyl-L-alanine methyl ester was found to be an excellent esteratic substrate for elastase. The activity of elastase towards this substrate is independent of pH between pH 8 and 10 and is unaffected by acetylation of the enzyme's N-terminal valyl α-amino group.

Introduction

The influence of the state of ionization of an N-terminal isoleucyl α-amino group on the activity and conformation of chymotrypsin is well supported by physical and chemical evidence (Oppenheimer et al., 1966; Himoe et al., 1967; Mathews et al., 1967; Sigler et al., 1968). Gertler and Hofmann (1967) have interpreted their data on the inactivation of elastase by nitrous acid as indicating an N-terminal valyl residue of elastase has a similar influence on elastase activity but this evidence is not clear-cut. Bender and Marshall (1968) concluded that the inhibition of elastase activity at extremely high pH values was due to the deprotonation of an N-terminal α-amino group and tentatively assigned a pK_a of 10.5-11 to the valyl α-amino group. However, an investigation of the ionization constants and reactivities of the amino groups of elastase (Kaplan and Hartley, 1969) gave a value of 9.7 for the pK_a of the α-amino group of the terminal valyl residue; no correlation of optical rotation with the state of ionization of this group could be demonstrated. These results prompted a reinvestigation of the role of the

amino terminal residue of elastase. The investigation was facilitated by the finding that N-benzoyl-L-alanine methyl ester, an excellent substrate for the α-lytic protease of Sorangium sp., (vide infra) is an equally good substrate for elastase.

Materials and Methods

Porcine elastase was prepared from Trypsin 1-300 (Nutrition Biochemicals Corp.) at the Laboratory of Molecular Biology, Cambridge by the method of Lewis et al. (1956) as modified by Smillie and Hartley (1966). N-benzoyl-L-alanine methyl ester was prepared by the method of Hein and Niemann (1962). Elastin was obtained from Worthington Biochemical Corporation and carboxymethylated B chain of insulin from Mann Research Laboratories, Inc.

Enzyme concentrations were determined from the amino acid analyses of a master stock solution and the known amino acid composition of the enzyme (Gertler and Hofmann, 1967). The relationship between molarity and absorbance was estimated to be: $[E] = 2.06 \times 10^{-5} \times A_{280}^{1cm}$ where [E] is the molar concentration of elastase and A_{280}^{1cm} is the absorbance at 280 mμ with glass-distilled water as solvent. Esterase activity was measured in a pH-stat and elastolytic activity was measured according to the method of Sachar et al. (1955).

Acetylated elastase was prepared in a pH-stat maintained at pH 7.5. Acetic anhydride was added to a solution of elastase (0.5 mg/ml) over a period of approximately fifteen minutes, until the final solution was 0.3% ($^{v}/v$) in acetic anhydride. As the freeze dried acetylated enzyme dissolved with difficulty, the acetylation mixture was used directly.

Results

Acetylated elastase was homogeneous on electrophoresis in cellulose acetate; its mobility at pH 5 was approximately 75% of that of native elastase (Figure 1). Analysis by the 2,4-dinitrofluorobenzene method using the procedure described by Oppenheimer et al. (1966) and by the cyanate method (Stark and Smyth, 1963) indicated that the α-amino group of N-terminal valine and all the ε-amino groups of lysine residues were completely acetylated.

The effect of pH on the hydrolysis of N-benzoyl-L-ala-

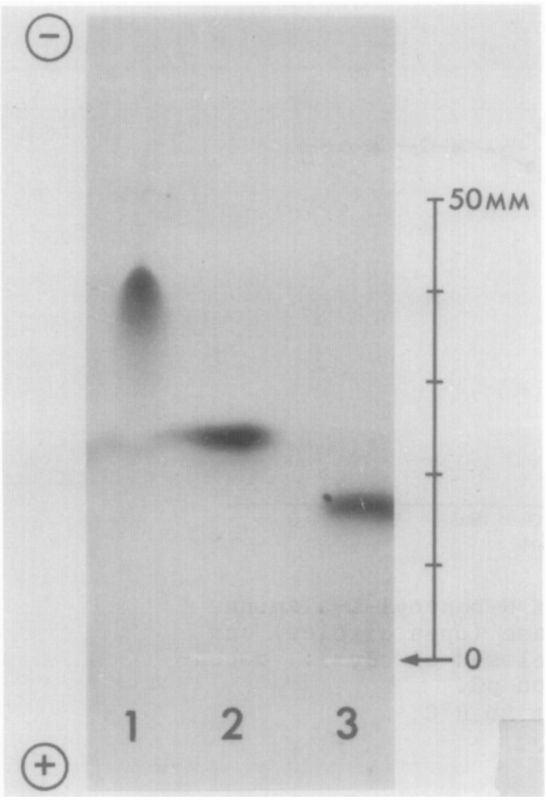

Fig. 1. Cellulose acetate ionogram of egg-white lysozyme (1), as a marker; elastase (2); acetylated elastase (3). Indicator: Ponceau S; buffer: 0.1 M sodium acetate - acetic acid pH-5.0; voltage gradient: 10 volts/cm; time: 180 min.

nine methyl ester by native elastase and acetylated elastase is shown in figure 2. The acetylated enzyme has essentially the same esteratic activity as the native enzyme and the activity of neither enzyme is influenced by pH between pH 8 and 10. These observations are not consistent with a dependence of catalytic activity on the state of ionization of α- or ϵ-amino groups. At lower pH the slope of the log k_{cat}/K_m function is 1, indicating the involvement of a single ionizing group with a pK_a of 6.5, presumably a histidine residue, in the catalytic mechanism. K_m for the reaction with native elastase at pH 8.0 was estimated to be 19.3 mM and k_{cat} to be 12.3 sec^{-1}.

So far as could be judged from a comparison of ionograms of digests of the carboxymethylated B chain of insulin by native elastase and acetylated elastase, acetylation does not alter the specificity of elastase. However, acetylation does reduce elastolytic activity by approximately 50% under the assay conditions used.

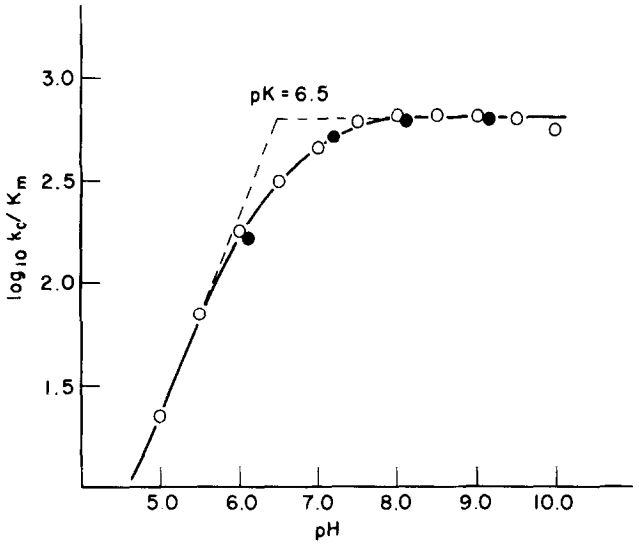

Fig. 2. Hydrolysis of N-benzoyl-L-alanine methyl ester by elastase (open circles) and acetylated elastase (closed circles): dependence of log k_{cat}/K_m on pH.
Solvent: 0.10N KCl at 25.0°C.

Discussion

The X-ray crystallographic structure of chymotrypsin (Mathews et al., 1967) has demonstrated that an ionic bond exists between the deprotonated carboxyl group of aspartic acid 194 and the protonated α-amino group of N-terminal isoleucine. It has been postulated (Sigler et al., 1968) that on ionization of the N-terminal amino group, the carboxylate ion is left in an environment of low dielectric constant and moves to a more polar environment thus destroying the conformational integrity of the active site. Elastase and chymotrypsin are homologous enzymes (Hartley et al., 1965) and elastase is therefore expected to have an activation mechanism and tertiary structure similar to that of chymotrypsin. However, its N-terminal amino group cannot influence activity in the same manner as that of chymotrypsin and it remains for a comparison of the X-ray crystallographic structures of the two enzymes to determine the reasons for this difference.

The above evidence, while indicating a difference between elastase and other pancreatic serine proteases, adds another

point of similarity between elastase and the α-lytic protease of Sorangium sp. This bacterial protease matches elastase in specificity (Whitaker et al., 1965), in its sequences around its active serine and histidine residues (Whitaker et al., 1966; Smillie and Whitaker, 1967), in its reaction mechanism (Kaplan and Whitaker, 1969) and in its esteratic activity being unaffected by acetylation of the enzyme or by exposure to alkaline pH's up to pH 10 (Kaplan and Whitaker, 1967). Other parallels between these two enzymes will be discussed in a future communication.

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