An N-Acetylmuramidase Induced by PL-1 Phage Infection of Lactobacillus casei

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A lytic enzyme was isolated and purified from PL-1 phage-induced lysates of the host Lactobacillus casei ATCC 27092. The molecular weight of the enzyme was about 30000. Maximum activity on the lysis of the host cell walls occurred at pH 6.0-6.5 and at 45 °C. The enzyme activity was inhibited by heavy metal ions, SH- and serine-enzyme inhibitors and o-phenanthroline. The reducing end of the enzymic digest was muramic acid and the enzyme was considered to be an endo-N-acetylmuramidase. However, the enzyme differed from the other known N-acetylmuramidases including hen’s egg-white lysozyme in several enzymic properties.

INTRODUCTION

Bacteriolytic enzymes, which are implicated in many biologically important phenomena, have been found to be useful tools for elucidating the surface structure of bacteria. However, there appears to be less information on bacteriolytic enzymes acting on Lactobacillus sp. than those acting on other bacteria. We have, therefore, examined PL-1 phage lysates for such bacteriolytic enzymes, since this phage specifically attacked these bacteria. As a result, a bacteriolytic enzyme was found in the phage lysates and identified as an N-acetylmuramidase (mucoprotein N-acetylmuramoyl hydrolase). Until now, besides egg-white lysozyme, N-acetylmuramidase from many origins has been purified and characterized (for reviews, see Imoto et al., 1972; Tsuru, 1977; Rogers, 1979). However, the enzyme in PL-1 phage lysates differed in several properties from all these enzymes. In this paper, we describe the isolation, purification and some characteristics of this enzyme.

METHODS

Phage PL-1 and its host Lactobacillus casei ATCC 27092, described by Watanabe et al. (1970, 1979) were used. Cell walls and the peptidoglycan and polysaccharide fractions were prepared as described by Ishibashi et al. (1982). Propagation of these organisms, their assay and other related methods were all described in the above papers.

Bacteriolytic activity was assayed turbidimetrically by incubating mixtures (final volume 3 ml, initial OD_{660} = about 0.35) of purified walls and enzyme at 37 °C in 0.01 M-phosphate buffer, pH 6-5. The decrease in OD_{660} of the incubation mixture was followed at 5 min intervals. One unit of bacteriolytic activity was defined as the amount of enzyme that gave a decrease in OD of 0.001 min⁻¹ during the initial period of linear decrease: unit = OD_{660} x 1000 min⁻¹. Specific activity was expressed as units (mg protein)⁻¹. The protein content was determined by the Lowry method, using bovine serum albumin as the standard.

The release of free reducing groups was determined by the method of Imoto & Yagishita (1971) and total amino groups by that of Yemm & Cocking (1955).

RESULTS AND DISCUSSION

When cells of Lactobacillus casei in the early-exponential phase of growth were incubated with PL-1 phages at a m.o.i. of 0.5 in MR medium, pH 6-0, at 37 °C, the cell wall-lytic enzyme activity began to increase when cell-lysis was first observed, approximately 3 h after the phage addition.
The maximum activity (about 20 units ml\(^{-1}\)) was observed at 9 h of incubation when the lysis was complete. This bacteriolytic activity was not detected either in the culture supernate of phage-uninfected host cells or in the extract of mechanically-disrupted cells. Therefore, this bacteriolytic activity was considered to result from the action of a PL-1-coded enzyme.

After removing cell debris by centrifuging the incubation mixture, the supernate was salted out with ammonium sulphate at 60\% saturation. After storage overnight at 2 °C, the precipitate was collected by centrifugation, dissolved in 0·01 M-phosphate buffer, pH 6·5, and dialysed against the same buffer to remove salt. The dialysate was applied to a DEAE-Sephacel column equilibrated with 0·01 M-Tris/HCl buffer, pH 7·2, and, after washing with the same buffer, the column was eluted with 0·4 M-NaCl and fractions (4 ml) were collected. Those containing the lytic activity were pooled, concentrated and subjected to rechromatography. The fractions containing lytic enzyme activity were then applied to a Sephadex G-75 column, eluted with 0·01 M-Tris/HCl buffer, pH 7·2, and again appropriate fractions were subjected to gel filtration as above to obtain a single protein fraction coinciding with lytic activity. The final product had specific activity of 2·07 \(\times 10^3\) units (mg protein\(^{-1}\)). Samples of the pooled enzyme were examined by SDS-PAGE. The enzyme gave only one band of protein having an apparent molecular weight of 30000, which was the same value as that obtained by gel filtration on TOYO pearl HW-50 (cross-linked polyvinyl: Toyo-Sohda Co., Japan). Thus, the enzyme was found to be composed of a single subunit.

The enzyme activity had a pH optimum of about 6·0-6·5 and an optimum temperature of 45 °C. Enzyme activity was inhibited significantly by metal ions such as Cu\(^{2+}\), Fe\(^{3+}\) and Cd\(^{2+}\) at a concentration of 1 mM, but it was not affected by other metal ions such as Mg\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\) and Zn\(^{2+}\) at the same concentration and ionic strength (NaCl from 0 to 100 mM). The enzyme activity was strongly inhibited by SH-enzyme inhibitors such as monooiodoacetate (1 mM) and p-chloromercuribenzoate (10 mM) and by serine-enzyme inhibitors such as diisopropylfluorophosphate (1 mM) and phenylsulphonyl fluoride (10 mM). o-Phenanthroline (0·1 mM) was the most effective inhibitor among the compounds tested.

The enzyme hydrolysed the peptidoglycan fractions isolated from the walls of \textit{Lactobacillus casei}, but did not degrade the accessory polysaccharide fractions of these walls. Digestion of the peptidoglycan was accompanied by the release of reducing groups without a concomitant increase of amino groups. The reducing end was shown to be N-acetylmuramic acid by boro-hydride reduction (Tsugita \textit{et al.}, 1968). These results indicated that PL-1 phage-induced lytic enzyme was an N-acetylmuramidase, which cleaved \(\beta\)-1,4-linkages between N-acetylmuramic acid and N-acetylglucosamine. No evidence was obtained for the presence of either N-acetylmuramyl-L-alanine amidase or endopeptidase activity.

Lytic action spectra of PL-1 phage-induced lytic enzyme presented here were also distinguishable from those of hen’s egg-white lysozyme. When ethyleneglycol chitin (0·05\%\%), which is used widely as a good substrate of egg-white lysozyme, was incubated with PL-1 phage-induced lytic enzyme (30 \(\mu\)g ml\(^{-1}\)) in 0·1 M-acetate buffer, pH 4·5, at 40 °C, no free reducing groups were released, showing that this enzyme was inactive on this polymer. Egg-white lysozyme at the same concentration released about 200 nmol of reducing groups after 40 min incubation. On the other hand, egg-white lysozyme (50 \(\mu\)g ml\(^{-1}\)) dissolved the peptidoglycan of \textit{Lactobacillus casei} (initial OD\(_{660}\) = 0·3) only slowly and partially in 0·01 M-Tris/HCl buffer, pH 7·2, at 37 °C, while, at the same concentration, PL-1 phage-induced lytic enzyme rapidly dissolved these walls. Many factors such as the presence or absence of acetyl groups on amino and hydroxy groups, the presence of accessory polymers such as teichoic acids and polysaccharides, and the degree of cross-linking in the peptide portion have been reported to influence the substrate specificity of bacteriolytic enzymes (Strominger & Ghuysen, 1967; Tsuru, 1983). The differences in the action of PL-1 phage-induced lytic enzyme and hen’s egg-white lysozyme are currently being investigated.

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Phage-induced lytic enzyme of *L. casei*

REFERENCES


