Lysozymes from Bacteriophages T3 and T5

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Lysozymes produced in host cells infected with bacteriophages T3 and T5 were found to have the same enzymatic specificity toward the peptidoglycan from Escherichia coli as T7 phage lysozyme, which has been shown to be an N-acetylmuramyl-L-alanine amidase.

It is well known that lytic enzymes are produced in cells infected with bacteriophages. However, the enzymatic specificities of these lytic enzymes vary depending upon the bacteriophage. The lytic enzymes, lysozymes, produced by T2 and T4 phages, are N-acetylmuramidases as in hen egg white lysozyme (4, 7), and λ phage produces an endopeptidase upon infection (8). More recently we have shown that T7 phage lysozyme is an N-acetylmuramidase (1).

In the present paper, we have further examined the enzymatic specificities of lytic enzymes produced in Escherichia coli infected with bacteriophage T3 or T5. It was revealed that both bacteriophages produce enzymes which have the same specificity toward the peptidoglycan from E. coli as T7 phage lysozyme, an N-acetylmuramidase.

The present method to determine the specificities of lytic enzymes has been developed in our laboratory and is based on the fact that a lipoprotein covalently linked to the peptidoglycan can be released in different fashions according to the enzymatic specificities (2). When the E. coli peptidoglycan is labeled with N-acetyl[14C]glucosamine and [3H]arginine, the N-acetylglycosamine is incorporated into the glycan portion of the peptidoglycan and the arginine is incorporated into the lipoprotein mentioned above.

The peptidoglycan labeled with N-acetyl[14C]glucosamine and [3H]arginine was prepared as described previously (2) and digested with T3 or T5 lysates. The [3H]-labeled lipoprotein was released from the peptidoglycan and became soluble in a sodium dodecyl sulfate solution. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the whole digest, the lipoprotein formed a peak in the gel at a position of molecular weight about 8,500 for both digests by T3 and T5 lysates (Fig. 1 and 2).

On the other hand, only less than 2% of total 14C radioactivity due to the glycan chains of the peptidoglycan is incorporated in the peak area of the lipoprotein (Fig. 1 and 2). The remaining 14C radioactivity spreads through the gel. These patterns, seen in both Fig. 1 and 2, are almost identical with that observed for T7 lysate in the previous paper (2). These results indicate that both T3 and T5 lysates contain N-acetylmuramyl-L-alanine amidases as does T7 lysate. In contrast, in the case of digestion with T4 phage lysozyme, an N-acetylmurami-
Since it is known that bacteriophage T5 is quite different from bacteriophages T3 and T7 (5), we further examined the lytic enzyme activity in T5 lysate with the use of the peptidoglycan labeled with \[^{3}H\]diaminopimelic acid and \[^{14}C\]arginine, prepared as described previously (2; Fig. 3). The peptidoglycan was digested with T5 lysate and subjected to polyacrylamide gel electrophoresis. About 10% of total \[^{3}H\]diaminopimelic acid label comigrates with the lipoprotein peak labeled with \[^{14}C\]arginine at molecular weight of 8,900, and the remaining label forms a peak between the molecular weight standards d and e. This result is also identical with the gel pattern obtained with T7 lysate. (2).

The present results show that lytic enzymes produced in cells infected with T3 or T7 phages have the same enzymatic specificity towards the E. coli peptidoglycan as T7 lysozyme, which has been found to be an N-acetylmuramyl-L-alanine amidase (2).

T3 and T7 phages are known to be closely related to each other, whereas T5 phage is quite different from T3 and T7 phages in many respects such as size and the structure of its DNA and its mode of infection (5). Nevertheless, T5 as well as T3 and T7 phages seem to produce N-acetylmuramyl-L-alanine amidases in the infected cells.

In the case of T7 (thus probably also T3), it has been shown that lysozyme has a role in DNA metabolism rather than lysis of the infected cells (S. Silberstein, M. Inouye, and F. W. Studier, J. Mol. Biol., in press). On the other hand, T5 lysozyme has been shown to be one of the later enzymes produced in the infected cells (6). At present it is not known whether T5 lysozyme is also involved in DNA metabolism or just in lysis of the cell.

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LITERATURE CITED


