

NOTES

Bacteriophage T3- and T7-directed Deoxyribonucleases

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The net content of deoxyribonucleic acid (DNA) of *Escherichia coli* cells infected with the wild types of T3 or T7 does not change markedly throughout the latent period (A. B. Pardee and I. Williams, *Ann. Inst. Pasteur* **84**:147, 1953; F. W. Putnam et al., *J. Biol. Chem.* **199**:177, 1952). However, we recently described the breakdown of host DNA by "early" amber mutants of two complementation groups of both T3 and T7 (R. Hausmann and B. Gomez, *J. Virol.* **1**:779, 1967). These combined observations suggest that, upon infection with the wild types of these phages, breakdown of host DNA also occurs but is masked by the simultaneous buildup of phage-specific DNA. Thus, the question arises whether this breakdown is related to the synthesis of phage-specific nucleases or whether phage infection renders the host DNA accessible to pre-existing nucleases of the host. We think that this latter view is favored by the findings of A. B. Pardee and I. Williams (*Ann. Inst. Pasteur* **84**:147, 1953), who reported a relatively slight increase in deoxyribonuclease activity in host cells after infection with T3. We used different assay conditions and found that pronounced increases in deoxyribonuclease activity occurred shortly after infection with phage T3 or T7.

To assay deoxyribonuclease activity in extracts of phage-infected *E. coli* B, we determined the amount of radioactivity released in acid-soluble form from ³H-thymidine-labeled *E. coli* B DNA (A. Weissbach and D. Korn, *J. Biol. Chem.* **238**:3383, 1963). Procedures of phage-infection, harvesting of cells, and preparation of cell-free extracts were similar to those described by M. Gold et al. (*Proc. Natl. Acad. Sci. U.S.* **52**:292, 1964). Assays were first conducted with incubation mixtures buffered at various pH values, and both native and alkali-denatured DNA preparations were used as substrates. We used extracts of cells harvested 10 min after infection with T3 or T7 as an enzyme source. Relative to uninfected cells, maximal increases in deoxyribonuclease

activity, as compared to native DNA, were about 1.3-fold for T3-infected cells and about fourfold for T7-infected cells; as compared to denatured DNA, the relative increases were about four- and sixfold, respectively (Fig. 1). Similar results were

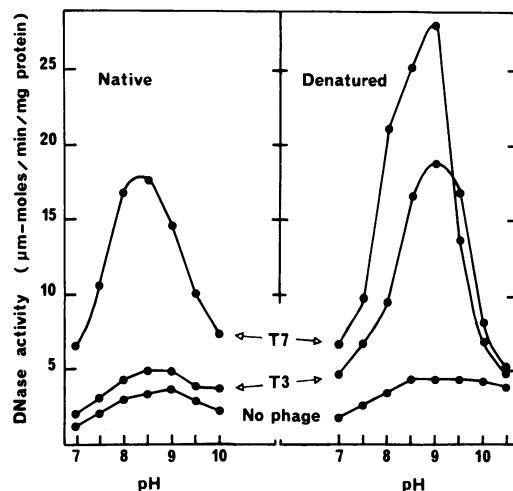


FIG. 1. Curves (pH) of deoxyribonuclease activity in cell-free extracts of *Escherichia coli* B harvested before or 10 min after infection with phage T3 or T7. Incubation mixtures were buffered with tris(hydroxymethyl)aminomethane (Tris)-ethanolamine, and the reactions were allowed to proceed for 20 min at 37°C. Enzyme activity is given as μ moles of DNA bases released in acid-soluble form (per min, per mg of protein in the incubation mixture) from the ³H-thymidine-labeled *E. coli* DNA substrate. At left: native DNA was used as the substrate; at right: the DNA substrate was previously denatured (pH 13, for 10 min at room temperature) and then neutralized.

obtained when T3 or T7 DNA was used as a substrate.

The increases in deoxyribonuclease activity as a function of time after phage infection were then measured (Fig. 2). In view of the previous find-

ings, assays were conducted at pH 9, and denatured DNA was used as the substrate. Besides the wild types of T3 and T7, two "early" amber mutants, representing the complementation groups DO-A and DO-B in each of these phages, were also assayed. We showed that mutants of both of these groups were unable to promote phage-directed DNA synthesis and that mutants of group DO-B broke down the host DNA at a much higher rate and to a greater extent than did homologous mutants of group DO-A (R. Hausmann and B. Gomez, *J. Virol.* 1:779, 1967). It is noteworthy that the rates of increase in deoxyribonuclease activity after infection (Fig. 2) roughly paralleled the previously observed breakdown rates of host DNA (R. Hausmann and B.

Gomez, *J. Virol.* 1:779, 1967); the times of increase in deoxyribonuclease activity and the onset of breakdown of host DNA also coincided. This suggested that the phage-directed deoxyribonucleases of T3 and T7 may be involved in the breakdown of host DNA. When chloramphenicol (80 $\mu\text{g}/\text{ml}$) was added at the same time as the phage inoculum, an increase in deoxyribonuclease activity was totally inhibited. When added 4 min after the phage inoculum, chloramphenicol stopped the increases at 15 and 35% of the maximal levels for T3 and T7, respectively.

Chloramphenicol sensitivity, differences in relative affinities toward native and denatured DNA, and differences in pH curves support the hypothesis that increases in deoxyribonuclease activity after T3 or T7 infection are due to the synthesis of new enzymes coded for by the phage genomes. The kinetics of these increases, showing the characteristic pattern of "early enzyme" synthesis, also support this notion.

Virus-directed nucleases have been described for many bacteriophages, such as those of the T-even and T5 (A. B. Stone and K. Burton, *Biochem. J.* 85:600, 1962) and of γ (D. Korn and A. Weissbach, *J. Biol. Chem.* 238:3390, 1963). Their function and mechanism of action, however, are not well understood. The most straightforward explanation would be that phage-directed nucleases bring about the breakdown of the host DNA, thus making the nucleotides available for the synthesis of phage-specific DNA. However, in the case of T5, the phage-directed deoxyribonuclease activity appeared only about 10 min after infection, at a time when the host DNA was already broken down extensively. (A. B. Stone and K. Burton, *Biochem. J.* 85:600, 1962). In phage λ , the opposite was observed: phage-directed deoxyribonuclease activity appeared after infection or induction (D. Korn and A. Weissbach, *J. Biol. Chem.* 238:3390, 1963) but extensive breakdown of host DNA did not seem to occur (L. Siminovitch, *Ann. Inst. Pasteur* 84:265, 1953). These observations suggest that phage-directed nucleases may have other biological functions (I. R. Lehman, *Ann. Rev. Biochem.* 36:645, 1967), and detailed investigation of T3- and T7-directed deoxyribonucleases seems indicated.

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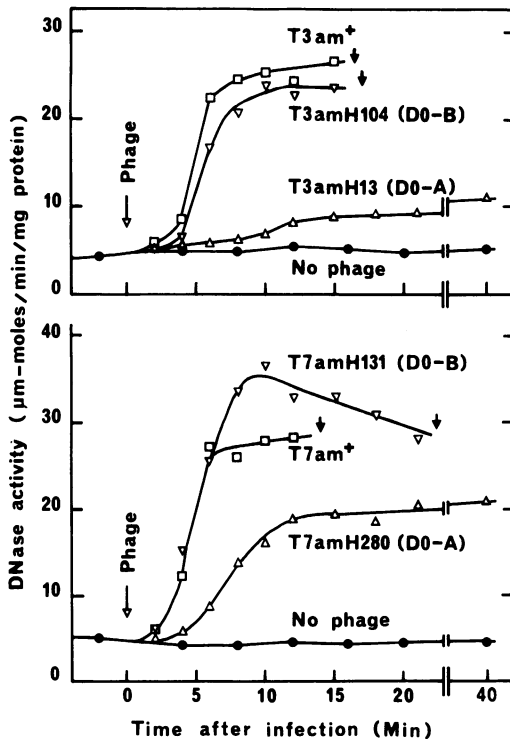


FIG. 2. Phage-directed increase in deoxyribonuclease activity after infection of *Escherichia coli* B (nonpermissive for amber mutants) with the wild-types of T3 or T7 or with amber mutants defective in phage-directed DNA synthesis. Cell-free extracts of bacteria harvested at various times after infection were used as an enzyme source. Assays were performed at pH 9, and alkali-denatured *E. coli* DNA was used as the substrate. Black arrows indicate the time of visible lysis.