Requirement for a Functional Host Cell Autolytic Enzyme System for Lysis of *Escherichia coli* by Bacteriophage φX174

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*Escherichia coli* VC30 is a temperature-sensitive mutant which is defective in autolysis. Strain VC30 lyses at 30°C when treated with β-lactam antibiotics or d-cycloserine or when deprived of diaminopimelic acid. The same treatments inhibit growth of the mutant at 42°C but do not cause lysis. Strain VC30 was used here to investigate the mechanism of host cell lysis induced by bacteriophage φX174. Strain VC30 was transformed with plasmid pUH12, which carries the cloned lysis gene (gene E) of phage φX174 under the control of the lac operator-promoter, and with plasmid pMC7, which encodes the lac repressor to keep the E gene silent. Infection of strain VC30(pUH12)(pMC7) with phage φX174 culminated in lysis at 30°C. At 42°C, intracellular phage development was normal, but lysis did not occur unless a temperature downshift to 30°C was imposed. Similarly, induction of the cloned φX174 gene E with isopropyl-β-d-thiogalactoside resulted in lysis at 30°C but not at 42°C. Temperature downshift of the induced culture to 30°C resulted in lysis even in the presence of chloramphenicol. These results indicate that host cell lysis by phage φX174 is dependent on a functional cellular autolytic enzyme system.

Bacteriophage φX174 is a small phage with a single-stranded DNA genome which has been completely sequenced (19). Lysis of *Escherichia coli* after infection with phage φX174 requires the product of the phage gene, designated E, but the gene E product apparently is not essential for the formation of mature progeny phage (12). Gene E of phage φX174 has been recently cloned, and it has indeed been demonstrated that the expression of the cloned gene is sufficient to cause lysis of *E. coli* (10, 21). The molecular weight of the gene E protein is ca. 10,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17) or by its amino acid composition deduced from the nucleotide sequence of the E gene (1). The gene E protein is apparently devoid of cell wall-degrading enzymatic activity (7, 16). This protein has been localized in the *E. coli* membrane (2). The appearance of the gene E protein during φX174 infection is associated with membrane alterations in the infected cells (15). The degradation of the peptidoglycan of infected cells, which is apparently catalyzed by cellular peptidoglycan endoglycosidase and endopeptidase activities, has also been observed during this period of the infection cycle (14). On the basis of these observations, it has been proposed that the gene E protein causes the lysis of φX174-infected cells by somehow inducing the activities of the cellular autolytic enzyme system (14), and the results reported here support this idea.

We have recently described (9) a mutant of *E. coli*, strain VC30 (thi lysA dapD ltyA), which carries a temperature-sensitive mutation in a gene we propose to designate as ltyA. The ltyA gene maps at 58 min on the *E. coli* linkage map and apparently encodes a protein involved in the regulation of the autolytic enzyme system (Harkness and Ishiyug, manuscript in preparation). Strain VC30 was transformed by procedures described previously (10) with plasmids pUH12 and pMC7. Plasmid pUH12 is a pUR222 derivative which carries the cloned gene E of phage φX174 placed under the control of the lac operator-promoter (10). Plasmid pMC7 is a ColE1 derivative which carries the lac repressor gene with the promoter mutation lacF (3, 4) and was necessary here to maintain the intracellular level of lac repressor high enough to prevent the expression of the E gene (11). Strain VC30(pUH12)(pMC7) was grown in a medium consisting of (per liter) 10 g of nutrient broth (Difco Laboratories), 5 g of yeast extract (Difco), 5 g of NaCl, and 50 mg of diaminopimelic acid. To ensure maintenance of plasmids pMC7 and pUH12, cells were grown in the presence of tetracycline (20 μg/ml) and ampicillin (200 μg/ml), respectively. In the experiments involving phage infections, CaCl2 was added to the medium to a final concentration of 10−2 M. Cultures were grown at the indicated temperatures in gyratory water bath shakers. The culture turbidity was determined with a Klett-Summerson colorimeter (green filter).

Strain VC30 exhibits a temperature-dependent defect in autolysis (9), and it was important to determine whether the introduction of pUH12 and pMC7 into this strain altered this phenotype. Figure 1a shows that a culture of strain VC30(pUH12)(pMC7) growing at 30°C lysed shortly after the addition of d-cycloserine. Furthermore, a temperature upshift from 30 to 42°C caused a reduction in the growth rate of VC30(pUH12)(pMC7). D-Cycloserine completely inhibited growth at 42°C but did not cause lysis. However, autolysis occurred when the d-cycloserine-treated culture was shifted down to 30°C. Similarly, when strain VC30(pUH12)(pMC7) was treated with β-lactam antibiotics or deprived of diaminopimelic acid, lysis occurred at 30°C but not at 42°C. These results indicate that the presence of plasmids pUH12 and pMC7 had no effect on the autolysis-defective phenotype of strain VC30.

The effects of phage φX174 infection on strain VC30(pUH12)(pMC7) are shown in Fig. 1b. At 30°C, the culture lysed ca. 45 min after infection with phage φX174 K9, a host-range mutant which has a high plating efficiency on *E. coli* K-12 strains (6). In contrast, φX174 infection at 42°C resulted in an immediate inhibition of growth, but no lysis occurred. Intracellular phage development, as determined by the method of Denhardt and Sinsheimer (5), appeared to be normal at 42°C (data not shown). Mature

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FIG. 1. Effects of p-cycloserine treatment (a), phage φX174 infection (b), and induction of the cloned φX174 gene E (c) on the temperature-sensitive, autolysis-defective mutant strain VC30(pUH12)(pMC7). Portions of cultures growing exponentially at 30°C (○) were shifted to 42°C (△). At the times indicated by the arrows in each panel, the following additions were made to portions of the 30°C cultures (●) and the 42°C cultures (▲): (a) p-cycloserine, 50 μg/ml; (b) φX174 K9 at a multiplicity of infection of 3; (c) IPTG, 5 × 10⁻³ M. In each experiment, the treated culture at 42°C was subjected to a temperature downshift to 30°C at the times indicated by the arrowheads. In (c), the temperature downshift was performed in the presence (●) and absence (▲) of chloramphenicol (100 μg/ml).

progeny phages were first detected at about 20 min postinfection. A maximum intracellular titer of 250 phage per cell was achieved at 30 min postinfection, and this remained unchanged for the duration of the experiment. A temperature downshift to 30°C resulted in lysis of the culture (Fig. 1b) and release of the phages (data not shown). These results indicate that φX174-induced lysis is dependent on a functional cellular autolytic enzyme system.

Figure 1c shows the effects of the expression of the cloned φX174 E gene on strain VC30(pUH12)(pMC7). At 30°C, lysis of the culture occurred about 55 min after the induction of gene E with 5 × 10⁻³ M isopropyl-β-D-thiogalactoside (IPTG). In contrast, growth was inhibited at 42°C, but no lysis occurred. The failure to lyse at 42°C was clearly not due to insufficient expression of gene E because lysis occurred shortly after the culture was subjected to a temperature downshift to 30°C even in the presence of chloramphenicol. Thus, the action of the gene E protein requires the function of cellular autolytic enzymes.

A culture of strain VC30(pUH12)(pMC7) was induced with IPTG at 30°C, and portions were then shifted to 42°C at the indicated times (Fig. 2). Two points are noteworthy: (i) First, the minimum time required for the action of the gene E protein in this system can be estimated if we consider that a period of 35 min at 42°C is required to inactivate the thermolabile lytA protein in strain VC30, i.e., to inactivate the autolytic enzyme system (9). The portions of the induced culture kept at 30°C for up to 20 min before upshift to 42°C were completely protected from lysis (Fig. 2, curves 1 to 3). On the other hand, cultures kept at 30°C for 30 min or more lysed upon upshift to 42°C, and the longer the incubation at 30°C, the greater was the rate and extent of lysis (Fig. 2, curves 4 to 7). For comparison, a culture kept at 30°C lysed 60 min after induction (curve 8). If an incubation of 35 min at 42°C is required to inactivate the autolysis system in strain VC30(pUH12)(pMC7) and since lysis occurred at 42°C only when induced cultures were kept at 30°C for at least 30 min before temperature upshift, we estimate that the minimum time required for the gene E protein to cause lysis is about 65 min after induction. Further interpretation of this data may be possible upon successful development of techniques to quantitatively follow the induction of gene E (Lubitz, unpub-

FIG. 2. Effect of temperature upshift to 42°C after induction of strain VC30(pUH12)(pMC7) at 30°C. A culture growing at 30°C (○) was induced with 5 × 10⁻³ M IPTG at the time indicated by the arrow. Portions of the culture were shifted to 42°C at 0 (curve 1), 10 (curve 2), 20 (curve 3), 30 (curve 4), 40 (curve 5), 50 (curve 6), and 60 min (curve 7) after addition of IPTG. Curve 8, induced culture left at 30°C; curve 9, a culture shifted to 42°C without IPTG.

lished data). (ii) It is also noteworthy that upshift of cultures induced with IPTG to 42°C resulted in an immediate inhibition of growth (compare induced cultures with the uninduced culture at 42°C [curve 9]; also Fig. 1c). IPTG did not affect the growth rate at 30°C up until the time of lysis. Furthermore, IPTG had no effect on the growth rate of the parent strain, VC30 (i.e., the strain lacking plasmids pUH12 and pMC7), at 42°C (data not shown). Thus, the growth-inhibitory effect of IPTG on strain VC30(pUH12)(pMC7) at 42°C was apparently in some way due to the expression of gene E.

In conclusion, these results suggest that the lysis of host cells by phage ϕX174 involves the interaction of the gene E lysis protein with the cellular autolytic enzyme system; but exactly how the gene E protein turns on the activities of the autolytic enzymes and how the timing of this event is regulated during the infection cycle are yet to be determined. It appears that the function of the lysis proteins of the bacteriophages MS2 (18) and QB (13, 20) and the cloacin DF13 plasmid (8) may be similar to that of the phage ϕX174 gene E protein.

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LITERATURE CITED