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Lysis of *Escherichia coli* after Infection with ϕX174 Depends on the Regulation of the Cellular Autolytic System

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The relationship between the rate of lysis of *Escherichia coli* infected with bacteriophage ϕX174 and the physiological state of the host bacteria was determined. The lysis rate was comparable to the growth rate only in cells grown in rich media, whereas in minimal medium it was much slower than the growth rate. Lysis of starved cells grown in minimal medium could not be induced by ϕX174 although progeny phages were produced. Lysis of *E. coli* provoked by expression of the cloned ϕX174 lysis gene could be prevented by $MgSO_4$ concentrations which also prevented lysis by induced autolysins whereas prevention of lysis of phage-infected *E. coli* needed much higher concentrations of $MgSO_4$. Prevention of lysis in the latter case did not re-establish viability of the infected cells whereas induction of the cloned ϕX174 lysis gene allowed continued multiplication in the presence of $MgSO_4$. Lysis of *E. coli* by expression of the cloned ϕX174 lysis gene was suppressed at pH 6·0 and could be turned on immediately upon upshift to pH 6·8. Phage-infected cells lysed at pH 6·0. At pH 8·0, lysis of *E. coli* by phage infection or by the cloned lysis gene product was suppressed. pH downshifts in both cases were not followed by lysis. The results suggest that the ϕX174 lysis gene product interacts in a reversible manner with the regulation of the autolytic system of *E. coli*.

INTRODUCTION

The release of progeny ϕX174 from *Escherichia coli* is accompanied by cellular dissolution (Hutchison & Sinsheimer, 1963; Denhardt & Sinsheimer, 1965). The presence of the phage gene *E* product (gpE) is necessary for the lysis since ϕX174 mutants with nonsense mutations in this gene fail to lyse the host bacterium (Hutchison & Sinsheimer, 1966). Expression of the cloned ϕX174 gene *E* is sufficient to achieve cellular disruption of *E. coli* and no other phage gene products are needed for cell lysis (Henrich *et al.*, 1982*a*; Young & Young, 1982), although ϕX174 mutants with defects in other genes than *E* exhibit inhibition or delay of lysis (Sinsheimer, 1968).

The mechanism by which gpE triggers cell lysis is not known. It is possible that lysis of the phage-infected cell differs from the lysis by the cloned ϕX174 lysis gene due to additional functions of the phage. On the other hand, lysis of ϕX174-infected bacteria is dependent not only on the genotype of the infecting phage but also on host functions, especially the physiological state of the bacteria (Denhardt & Sinsheimer, 1965). This could be a consequence of the mechanism by which ϕX174 causes lysis. Lubitz & Plapp (1980) suggested that ϕX174 induces lysis of *E. coli* by interfering with the autolytic system of the host.

The autolytic system of *E. coli* is highly dependent on the growth conditions of the bacteria. Cells grown in rich media can easily be induced to autolyse whereas cells grown in minimal medium with a poor carbon source or cells in the stationary growth phase cannot be induced at all (Leduc *et al.*, 1982). If ϕX174 interferes with the autolytic system of the host, lysis of the cells should also depend on factors determining the activity the autolytic system. To examine this hypothesis, the dependence of lysis of ϕX174-infected *E. coli* on the growth rate of the host

Abbreviations: DAP, *meso*-2,6-diaminopimelic acid; IPTG, isopropyl β-D-galactoside; MM, minimal medium.

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bacterium was studied. Autolysis of *E. coli* can be prevented by $MgSO_4$ (Leduc & Van Heijenoort, 1980); the inhibition of lysis by $MgSO_4$ of ϕX174-infected cells and cells which are lysed upon induction of the cloned lysis gene of the phage were therefore compared. Furthermore, the influence of the pH of the growth medium and the effect of high concentrations of β-lactam antibiotics on ϕX174-induced lysis of *E. coli* was studied. These investigations provide evidence that ϕX174-induced lysis of *E. coli* is closely coupled to lysis caused by the autolytic system.

METHODS

Bacteria, phages and plasmids. Escherichia coli W7 (Dap$^-$ Lys$^-$) requiring *meso*-2,6-diaminopimelic acid (DAP) and lysine was obtained from the culture collection of the Max-Planck-Institut für Biochemie, Martinsried, FRG. *Escherichia coli* C (*pheA*), requiring phenylalanine, was from the Phabagen Collection (PC 1363), Utrecht, Netherlands. *Escherichia coli* K12, strain CAi^{q1} [Δ(*lac pro*) *thi cap cya rpsL recA* F' *lacI*^{q1} *lac*$^+$ *pro*$^+$] was provided by U. Rüther, Institut für Genetik, Universität Köln, FRG.

Phage ϕX174 wild-type was from Miles and ϕX174 K9, a host range mutant of ϕX174 wt which plates with high efficiency on *E. coli* K12 strains (Dowell *et al.*, 1981) was provided by C. E. Dowell, University of Massachussetts, Amherst, Massachussetts, USA. ϕX174 W is a host range mutant derived from ϕX174 K9 by repeated single plaque selection of ϕX174 K9 grown on *E. coli* W7.

Plasmid pUH12 is a recombinant plasmid derived from plasmid pUR222 (Rüther *et al.*, 1981) carrying the 1007 bp *Alu*I fragment of ϕX174 RFI DNA, comprising gene *E* of ϕX174 under the control of the *lac* operator–promoter region (Henrich *et al.*, 1982*a*). Plasmid pUH123 is derived from pUH12 and carries the *lac i* gene and the tetracycline resistance genes as well as the ϕX174 sequence (Henrich *et al.*, 1983).

Growth conditions. Strains were grown either in LB medium containing (l^{-1}): 10 g tryptone, 5 g yeast extract and 10 g NaCl; or in minimal medium (MM) containing (l^{-1}): 10·5 g K_2HPO_4, 4·5 g KH_2PO_4, 1 g $(NH_4)_2SO_4$ and 0·5 g sodium citrate dihydrate. Stock solutions of 20% (w/v) glucose, succinate, glycerol and acetate were added to final concentrations of 0·2%. For growth of *E. coli* W7, final concentrations of DAP and lysine were 50 μg ml^{-1} and 20 μg ml^{-1}, respectively. For all experiments, bacteria were grown with aeration at 37 °C. Before phage infection, $CaCl_2$ was added to a final concentration of 2×10^{-3} M. Bacterial growth was measured at 600 nm in a Zeiss PM4 or Gilford spectrometer.

Shift of *E. coli* W7 grown in medium supplemented with DAP into DAP-free medium was performed by harvesting exponentially growing cells at an OD_{600} of 0·4. After centrifugation at room temperature cells were resuspended in twice the original volume of prewarmed medium without DAP and were further incubated.

Cells carrying plasmid pUH12 or pUH123 were grown in the presence of 200 μg ampicillin ml^{-1} and induced to lyse by adding isopropyl β-D-thiogalactoside (IPTG) to the culture to give a final concentration of 10^{-3} M.

Assay of bacteriophage. Plaque assays were performed on LB plates containing 10^{-3} M-$CaCl_2$. The $CaCl_2$ concentration in the top agar overlay was 10^{-3} M for ϕX174 wt, 5×10^{-3} M for ϕX174 W, and 10^{-2} M for ϕX174 K9. Assays were usually done at 37 °C and plaques were counted 5 h after inoculation.

Selection and purification of ϕX174 W. ϕX174 W is a host-range mutant of ϕX174 K9. ϕX174 K9 plates on *E. coli* W7 with an efficiency which is 10 times higher than ϕX174 wt, taking the titre of both phages on *E. coli* C as reference. Single plaques of ϕX174 K9 grown on *E. coli* W7 were selected by the size of the plaques and were repeatedly grown on *E. coli* W7, selecting each time for single, large plaques. Finally, a phage stock was derived which showed large clear plaques of *E coli* W7 and had an e.o.p. which was increased by a factor of 10 when compared to the e.o.p. of the parental phage ϕX174 K9.

High titre stocks of ϕX174 wt, ϕX174 K9 or ϕX174 W were obtained by inoculating early exponential cultures of *E. coli* C, *E. coli* K12 or *E. coli* W7, respectively, at a m.o.i. of 10^{-4} to 10^{-3} or with a single plaque. Cells were grown in LB with 5×10^{-3} M-$CaCl_2$ and vigorous aeration at 37 °C. Before complete lysis of the culture or shortly after (normally 2·5 to 3 h after addition of phages), cells and cell wall fragments were collected by centrifugation. The pellet was resuspended in 1/20 vol. 0·05 M-sodium tetraborate and the suspension was shaken gently overnight at 4 °C. The suspension was re-centrifuged and the supernatant saved. The titre of such a phage stock was usually 10^{11} to 10^{12} p.f.u. ml^{-1}.

For characterization of the DNA of ϕX174 W, phages were banded in $CsCl_2$ and RFI DNA of infected cells was extracted and digested with *Alu*I as described (Henrich *et al.*, 1982*b*). The *Alu*I restriction patterns of ϕX174 W and ϕX174 wt were identical.

Artificial lysis of E. coli and determination of intracellular phages. Premature lysis of ϕX174-infected *E. coli* was performed by a modification of the method of Denhardt & Sinsheimer (1965). Briefly, at various times after infection, 1 ml samples were withdrawn from the infected culture and immediately centrifuged in an Eppendorf microcentrifuge for 2 min. The supernatant was discarded, and the pellet was washed once with ice-cold 10 mM-Tris/HCl, 50 mM-EDTA, pH 8·0. The pellet was frozen in liquid nitrogen and kept frozen until the end of the experiment. Cells were thawed by adding 0·5 ml 50 mM-Tris/HCl, 50 mM-EDTA, pH 8·0. After addition of 50 μl

lysozyme (10 mg ml^{-1}) the samples were kept for 30 min on ice and then 5 min at 37 °C. Afterwards the samples were frozen and thawed three times and centrifuged in an Eppendorf centrifuge for 4 min. Samples of the supernatant were taken and diluted serially in 0·05 M-sodium tetraborate. Plaque assays were performed as described.

RESULTS

Correlation between the growth rate of E. coli and the lysis rate of φX174-infected cells

In order to detect a correlation between growth and lysis, the growth rate of *E. coli* W7 was influenced by the nutritional composition of the medium. Fastest growth of *E. coli* W7 was in LB broth (Fig. 1) with a generation time of 30 min at 37 °C. The generation times in minimal medium supplemented with glucose, succinate, glycerol or acetate were 70, 90, 100 and 130 min, respectively.

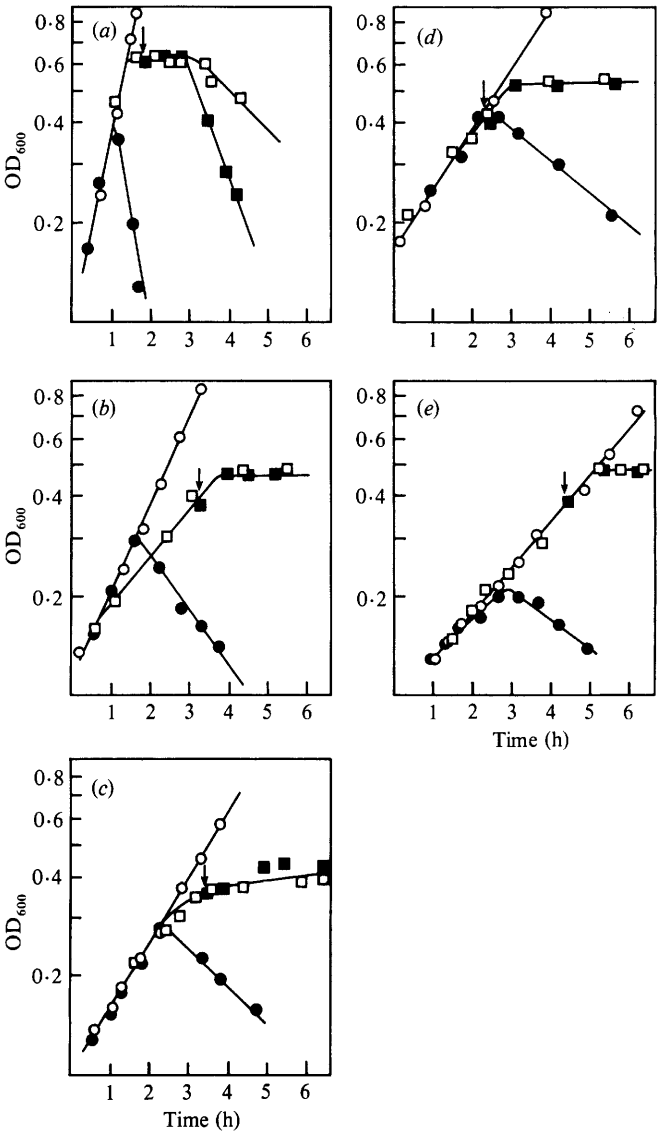

Fig. 1. Growth of φX174-infected and uninfected *E. coli* W7. Growth media were (*a*) LB broth, (*b*) MM with glucose, (*c*) MM with succinate, (*d*) MM with glycerol, (*e*) MM with acetate. ○, Uninfected culture; ●, φX174 W-infected culture, infection at time zero; □, uninfected culture after shift into DAP-free medium; ■, φX174 infection of DAP-deprived cells at the times indicated by the arrows.

Table 1. *Accumulation of mature progeny φX174 W in E. coli W7*

Escherichia coli W7 was grown in MM with glucose. During exponential growth cells were harvested at an OD_{600} of 0·4 by centrifugation and resuspended in 2 vol. prewarmed medium without DAP. At time zero cells were infected with φX174 W (m.o.i. = 1). Samples of 1 ml were withdrawn at times indicated and intracellular progeny phages were determined.

Time after infection (min)	10^{-7} × Phage titre after artificial lysis (p.f.u.)
20	0
30	37
40	80
50	166
60	190
70	322
80	392

As shown in Fig. 1, lysis of *E. coli* by φX174 depends strictly on the growth rate of the host. Lysis of φX174-infected cells depends on the activity of a system which is coupled to the growth rate of the bacterium. In slowly growing cells this system is much less active than in fast growing ones. Only in LB broth did the lysis rate of φX174-infected *E. coli* correspond to the growth rate. In minimal medium supplemented with glucose or succinate, the lysis rate was only slightly decreased, and in minimal medium supplemented with glycerol or acetate, the lysis rate was almost half the growth rate.

Retardation of lysis observed under these conditions was not the result of the inability of the infected cells to propagate and produce mature progeny phages, because the yield of progeny φX174 proceeded at a normal rate (Table 1). Under growth conditions where *E. coli* W7 was deprived of DAP and infected with φX174 W shortly before entering the stationary growth phase, spontaneous lysis occurred only in LB broth. The time of onset of phage lysis was similar to that of spontaneous lysis. The lysis rate of the infected sample, however, was faster than spontaneous lysis. When cells were grown in minimal medium, spontaneous lysis by exhaustion of DAP did not occur nor was lysis induced by the phage infection.

Influence of $MgSO_4$ on the lysis of φX174-infected E. coli

The variety of treatments by which autolysis of *E. coli* is induced share the common property that, regardless of the induction method used, 10^{-2} M-$MgSO_4$ efficiently inhibits the autolytic process (Leduc & Van Heijenoort, 1980; Leduc *et al.*, 1982). If we conclude from the experiments described above that lysis of *E. coli* by φX174 is brought about by induction of the autolytic system, one should be able to prevent lysis of φX174-infected cells by adding $MgSO_4$.

A final concentration of 10^{-2} M-$MgSO_4$ given either at the time of infection, in the middle of the infection cycle or just before onset of lysis did not prevent lysis of φX174-infected *E. coli* (Fig. 2). However, by raising the final concentration of $MgSO_4$ at the time of infection fivefold (Fig. 3), the onset of lysis was slightly retarded when compared to the untreated control. At 10^{-1} M-$MgSO_4$ a biphasic lysis curve resulted, indicating that half of the population of the sample lysed. At 2×10^{-1} M-$MgSO_4$, lysis was prevented in most of the cells (Fig. 3).

Lysis of E. coli by cloned φX174 gpE and inhibition of lysis by $MgSO_4$

The lysis behaviour of *E. coli* C harbouring plasmid pUH123 is shown in Fig. 4. This plasmid carries the *lac* repressor gene *i* in addition to the φX174-lysis function (Henrich *et al.*, 1983). The time from inactivation of the *lac* repressor by addition of IPTG until the start of cellular disruption in cells with pUH123 was 150 min. However, only a minute fraction of the cells were able to lyse if IPTG was added at the end of the exponential phase and there were no signs of lysis if IPTG was added later. This observation reinforces the conclusion that φX174-lysis is controlled by host functions coupled to the activity of the autolytic system (Leduc *et al.*, 1982).

In order to study the effect of $MgSO_4$ on gpE-induced lysis, *E. coli* CAi^{q1} carrying plasmid pUH12 was induced to lyse during exponential growth and $MgSO_4$ was added to a final

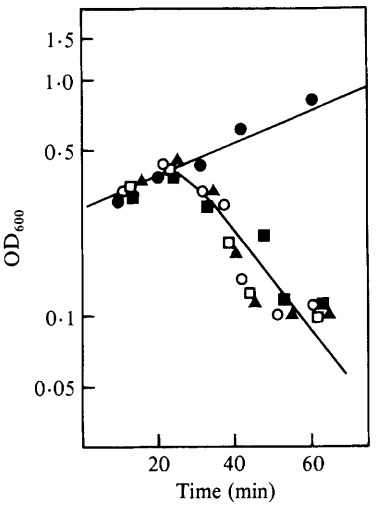

Fig. 2

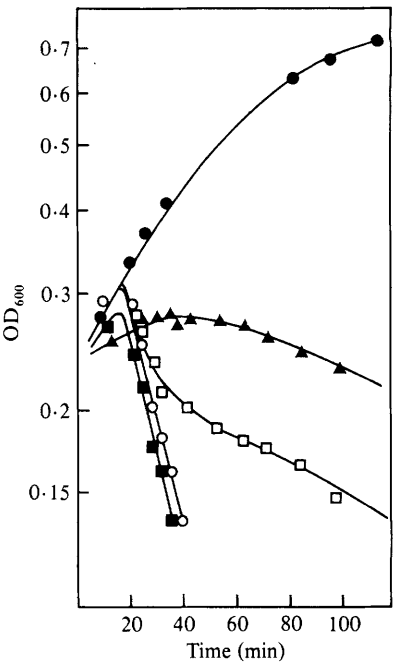

Fig. 3

Fig. 2. Effect of $MgSO_4$ added at various times after infection with φX174, on lysis of *E. coli* C. *Escherichia coli* C was grown in LB medium. In the early exponential growth phase at time zero one sample of the culture was kept as a control (●) and the rest was infected with φX174 wt (m.o.i. = 3) and divided into four samples. 10^{-2} M-$MgSO_4$ was added to the culture at time zero (■), 19 min after infection (□) and 36 min after infection (▲). ○, Untreated culture.

Fig. 3. Effect of concentration of $MgSO_4$ on lysis of *E. coli* after infection with φX174. *Escherichia coli* C was grown in LB medium. In the early exponential growth phase one sample of the culture was kept as a control and the rest of the culture was infected at time zero with φX174 wt (m.o.i. = 3) and divided into four samples. ●, Untreated control; ○, φX174-infected samples with no $MgSO_4$ added; ■, 5×10^{-2} M-$MgSO_4$; □, 10^{-1} M-$MgSO_4$; and ▲, 2×10^{-1} M-$MgSO_4$. $MgSO_4$ was added at the time of infection.

concentration of 10^{-2} M at the time of induction. The rate of lysis of the induced cells was retarded considerably. A concentration of 5×10^{-2} M-$MgSO_4$ was much more effective in inhibiting lysis and restoring growth (Fig. 5). Higher $MgSO_4$ concentrations did not produce other effects. Removing $MgSO_4$ from the cultures by sedimentation of the bacteria and resuspension in $MgSO_4$-free LB broth resulted in lysis.

The concentration of $MgSO_4$ needed to inhibit lysis caused by cloned gpE was much less than that for phage-infected cells. However, the most important difference from phage-infected cells is that $MgSO_4$-stabilized *E. coli* cells continue to multiply even in the presence of gpE (Table 2). The colony-forming ability was only slightly reduced under these conditions when compared to normal cells. The small decrease in colony forming units of cells committed to lysis by gpE and antagonized by $MgSO_4$ can be explained by the microscopical observation that these cells tended to grow as chains of six to eight times the unit length of *E. coli*. The bacteria, however, were only loosely held together as they could be separated easily into single cells during the dilution procedure for determination of c.f.u.

Influence of pH and antibiotics on φX174- or φX174 gpE-induced lysis of E. coli

In cultures of *Bacillus subtilis* and *Streptococcus pneumoniae* the bacteriolytic effect of β-lactam antibiotics could be suppressed if the pH of the culture was at 6·6 or below 6·0 (Lopez *et al.*, 1976). Growth at pH 5 protected *E. coli* against the lytic effect of these antibiotics whereas lysis

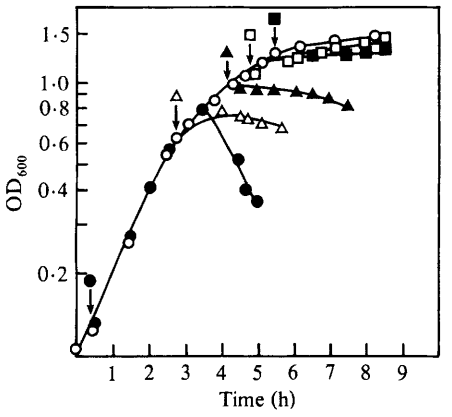

Fig. 4

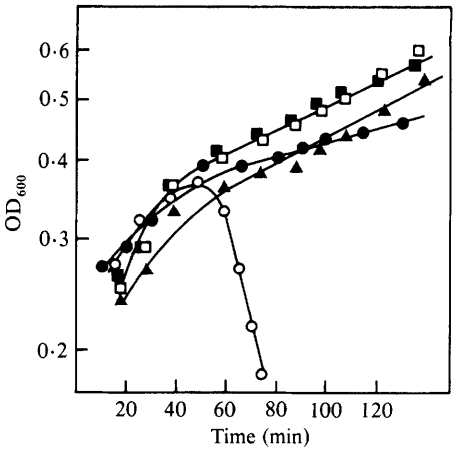

Fig. 5

Fig. 4. Lysis of *E. coli* C(pUH123) after induction of the ϕX174 lysis gene. *Escherichia coli* PC1363(pUH123) was grown in LB and, at the times indicated by arrows and symbols, IPTG (10^{-2} M final concentration) was added. ○, Untreated control.

Fig. 5. Effect of $MgSO_4$ on lysis of *E. coli* CAi^{q1}(pUH12) after induction of the ϕX174 lysis gene. *Escherichia coli* CAi^{q1}(pUH12) was grown in LB medium. In early exponential growth phase at time zero one sample of the culture was kept as a control (□), and to the rest lactose was added to give a final concentration of 5×10^{-3} M. $MgSO_4$ was added to give final concentrations of 5×10^{-2} M (●), 10^{-1} M (▲) and 2×10^{-1} M (■). ○, No $MgSO_4$ added.

Table 2. *Effect of gpE expression on E. coli viability*

All cells were grown in LB medium, strain CAi^{q1}(pUH12) with a final concentration of 5×10^{-2} M-$MgSO_4$. To one sample 5×10^{-3} M-lactose was added. *Escherichia coli* C was grown without $MgSO_4$ or lactose. Samples were diluted in LB broth and plated on LB agar.

	10^{-8} × No. of c.f.u. ml^{-1}		
OD_{600}	CAi^{q1}(pUH12)	CAi^{q1}(pUH12) + lactose	*E. coli* C
0·2	1·2	1	1·1
0·4	1·9	1·5	1·8
0·6	3	2·2	3
0·8	3·8	3·4	5
1·0	7·8	5	8·2

occurred at pH 7 or 7·5 (Goodell *et al.*, 1976). It seems, therefore, that the activity of autolytic murein hydrolases can be suppressed at low pH.

For plasmid-coded ϕX174 gpE we observed a similar dependence of its lytic action on the pH of the growth medium (Fig. 6). Between pH 7·5 and 6·6, lysis of *E. coli* occurred 120 min after induction of gpE with IPTG. However, if the culture was kept at pH 6·0 expression of gpE was followed by growth retardation without signs of lysis for several hours. Upshift of the growth medium from pH 6·0 to 6·8 was followed by immediate onset of lysis of the culture. Growth inhibition with no signs of lysis also occurred at pH 8·0 in cultures where cloned ϕX174 gpE was induced. However, pH-downshift of the medium 180 min after the beginning of growth inhibition was not followed by cellular lysis within 2 h.

In contrast to gpE-induced lysis, ϕX174-infected *E. coli* lysed in culture medium at pH 6·0 (Fig. 7*a*) and lysis inhibition was first detectable at pH 5·0 (Fig. 7*b*). Infection of the culture was carried out at pH 6·8 and infected bacteria were resuspended in medium at lower pH, 5 min after infection. Determination of intracellular progeny phages showed that phage development is not disturbed in cultures grown at pH 5·0. Surprisingly, at this pH culture growth was only

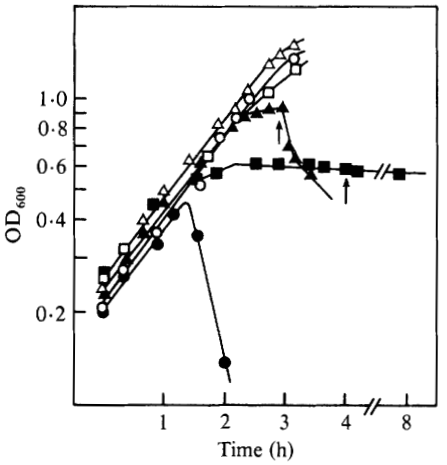

Fig. 6. Effect of pH on the response of *E. coli* C(pUH123) to gpE-induced lysis. *Escherichia coli* PC1363(pUH123) was grown in buffered LB medium. In early exponential growth phase at time zero the cultures were divided, and to one sample IPTG (5×10^{-3} M final concentration) was added to induce cell lysis. ○, Untreated culture at pH 6·6; ●, induced culture at pH 6·6; △, untreated culture at pH 6·0; ▲, induced culture at pH 6·0; □, untreated culture at pH 8·0; ■, induced culture at pH 8·0. The pH-shift to pH 6·6 is indicated by the arrows.

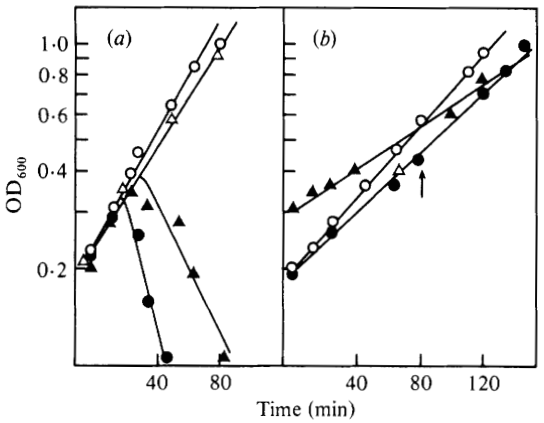

Fig. 7. Effect of pH on lysis of φX174-infected *E. coli*. *Escherichia coli C* was grown in buffered LB medium. In early exponential growth phase at time zero the cultures were divided and one sample was infected with φX174 wt (m.o.i. = 1). (*a*) ○, Uninfected sample, and ●, φX174-infected sample at pH 6·6; △, uninfected, and ▲, φX174-infected sample at pH 6·0. (*b*) ○, Uninfected and ●, φX174-infected sample at pH 8·0; the arrow indicates the pH-shift to pH 6·6; ▲, φX174-infected sample at pH 5·0.

retarded and not completely inhibited due to phage development. As already observed for gpE-induced lysis, lysis inhibition of φX174-infected cells also occurred at pH 8·0 (Fig. 7*b*) and, as for cultures kept at pH 5·0, this was without effect on the development of intracellular progeny phage.

Autolysis of *E. coli* triggered by low concentrations of β-lactams could be suppressed once initiated by high concentrations of certain β-lactams (Kitano & Tomasz, 1979). Cephaloridine, the most effective inhibitor of cell wall degradation, had no effect at concentrations up to 100 times MIC on lysis of *E. coli* initiated either by gpE alone or by infection with φX174 wt. With phage-infected cells a slight stimulation of lysis was observed under these conditions.

DISCUSSION

In the middle of the infection cycle of ϕX174-infected *E. coli*, a process is initiated which leads to rapid degradation of newly synthesized murein by at least two different enzymes, an endopeptidase and an endoglycosidase (Lubitz & Plapp, 1980). By cloning ϕX174 gene *E* (Henrich *et al.*, 1982*a*; Young & Young, 1982) it was shown that the action of gpE is sufficient to produce the lysis phenomenon exhibited with intact ϕX174. However, it is hard to imagine that gpE, which has a molecular mass of approximately 10 kDal (Barrell *et al.*, 1976; Pollock *et al.*, 1978), has both endopeptidase and endoglycosidase enzymatic activities. Efforts to isolate or characterize ϕX174-specific murolytic enzymes have failed so far (Fujimura & Kaesberg, 1962; Eigner *et al.*, 1963; Markert & Zillig, 1965).

The dependence of ϕX174-induced lysis of *E. coli* on host factors described in this communication provides evidence that the reaction resembles other systems where the autolytic system is induced artificially (Leduc & Van Heijenoort, 1980; Leduc *et al.*, 1982). In both cases lysis can only be achieved with exponentially growing cells because the activity of the enzymes responsible for this process is correlated with cell growth. Further support for this correlation comes from our unpublished observation that, in a mini-cell-producing strain, only mini-cells survive after expression of gpE, whereas parental cells are lysed completely. Lysis of *E. coli* by cloned gpE could be prevented by addition of $MgSO_4$ at a concentration which effectively inhibited artificially induced autolysis. The much higher concentration of $MgSO_4$ needed in ϕX174-infected cells to stabilize the cells against lysis reflects the more complex situation in phage-infected cells.

Further evidence for the activation of the autolytic system of *E. coli* by ϕX174 gpE comes from the investigation of the pH-dependence of the lysis process. The inhibition of lysis of *E. coli* caused by phage infection or gpE at low pH corresponds to the behaviour of *E. coli* after triggering the autolytic system by β-lactam antibiotics (Goodell *et al.*, 1976). In the latter case growth of *E. coli* at pH 5 protected the bacteria against the lytic effect of β-lactam antibiotics and, as we observed (Fig. 7), against lysis by ϕX174 infection. Resistance to gpE-induced lysis of *E. coli*, however, could be achieved at pH 6. Whether this difference is due to the induction of several autolytic enzymes in the case of phage infection and a single one in case of gpE remains to be investigated. Another reason for the different pH sensitivity of the autolytic process initiated either by gpE or ϕX174 infection may be due to the altered membrane composition which we observe in phage-infected cells (Lubitz *et al.*, 1981; Bläsi *et al.*, 1983).

More direct evidence that ϕX174 or cloned ϕX174 gpE cause lysis of *E. coli* by induction of the autolytic system comes from investigations using a temperature-sensitive autolysis-defective mutant of *E. coli* (Harkness & Ishiguro, 1983). In this case cultures of the mutant underwent lysis during β-lactam treatment, D-cycloserine treatment, ϕX174 infection or expression of gpE alone at 30 °C, whereas at 42 °C lysis was inhibited. Downshift to the permissive temperature in the presence of chloramphenicol was followed by lysis (W. Lubitz, R. E. Harkness and E. E. Ishiguro, unpublished). This behaviour resembles the mechanism proposed by Tomasz (1979) for β-lactam action and suggests that the mechanisms of ϕX174-induced lysis and cellular autolysis may be related. The possible interaction of gpE with regulatory functions of the autolytic system of *E. coli* strongly depends on cofactors produced by the host. Recently we isolated *E. coli* mutants which are no longer sensitive to gpE. The analysis of these mutants should provide new insights into the interplay between host and phage functions necessary to achieve autolysis of *E. coli*.

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