Factors in Lysis and Lysis Inhibition by Lambda Bacteriophage

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ABSTRACT

GROMAN, NEAL B. (University of Washington, Seattle). Factors in lysis and lysis inhibition by lambda bacteriophage. J. Bacteriol. 90:1563-1568. 1965.—Induced Escherichia coli strain K-12(λ112) exhibited lysis inhibition at 37°C but lysed at 44°C when incubated in LB medium lacking NaCl [LB — (NaCl)]. In LB medium containing NaCl, the temperatures for lysis and lysis inhibition were reversed. In contrast, induced K-12(λ) lysed under all of these conditions. At 37°C, the addition of NaCl to LB (NaCl) at various times after induction of K-12(λ112) restored lysis. The degree of lysis decreased the longer the addition was delayed, but partial restoration occurred as late as 150 min postinduction. At 44°C, the addition of salt at various times after induction restored lysis inhibition even after lysis had begun. An attempt was made to correlate the conditions for lysis and lysis inhibition with the behavior of λ112 endolysin. The enzymatic activities of λ and mutant λ112 endolysins were compared under various salt-temperature conditions. Both endolysins were progressively and equally inhibited by increasing concentrations of Na+, K+, and Li+ salts, and exhibited similar relative activities at 24 and 37°C. Both were stable at 37°C in the presence and absence of NaCl, and were inactivated at comparable rates at 44°C. The results indicate that the effect of salt and temperature on lysis and lysis inhibition cannot be explained by their direct effect on λ112 endolysin.

The relationship of endolysin synthesis to lysis by lambda phages was reported previously (Groman and Suzuki, 1963). Most of the observations supported the hypothesis that the timing and rate of endolysin synthesis determined the kinetics of the lytic process. However, under certain conditions, lysis inhibition was observed with mutant λ112 despite concurrent synthesis of large amounts of endolysin and phage. The effect of temperature on lysis and lysis inhibition was described previously (Groman, 1962). The present paper describes the influence of sodium chloride and other salts on lysis and lysis inhibition, and of salt and temperature on endolysin action.

MATERIALS AND METHODS

Bacteria and phages. Escherichia coli K-12, its lysogenic derivatives K-12(λ) and K-12(λ112), and a streptomycin-resistant mutant of K-12 used as indicator strain have all been described (Groman, 1962).

Media. The bacteria were grown in Trypticase Soy Yeast-extract (TSY) medium to the logarithmic growth phase. The medium contained Trypticase Soy base (BBL) supplemented with 2.5 g per liter each of dipotassium phosphate and glucose and 10 g per liter of yeast extract. The basic medium employed in the experiments was LB medium which contained 1% tryptone (Difco), 1% NaCl, 0.5% yeast extract, and 0.1% glucose at a final pH of 7.4. Most of the experiments were carried out in LB medium from which NaCl was omitted [LB — (NaCl)]. Other variations in the medium are described in Results. Induction of lysogenic strains was carried out in 0.01 M MgSO4, followed by a 1:1 dilution with double-strength medium of the appropriate type.

Methods. Phage assays, chloroform lysis, induction of lysogenic strains, and most endolysin assays were carried out as previously described (Groman and Suzuki, 1962, 1963). In certain experiments, the following minor modification of procedure was employed, which permitted assays of endolysin to be performed at 37°C as well as at room temperature. In this modification, the substrate, an acetone powder of K-12, was mixed with tri(hydroxymethyl)aminomethane buffer (0.05 M, pH 7) containing 0.0025 M MgSO4, to give an optical density of 1.0. Ethylenediaminetetraacetic acid (EDTA) was then added to a final concentration of 0.002 M, and the mixture was allowed to equilibrate for 5 min at the temperature of assay. Equal volumes of the substrate and appropriately diluted endolysin preparation were mixed, and optical-density changes were recorded for a 2- to 5-min period on a Gilford recording spectro-
However, the 44 at indicated by omission 44 at proved glucose inhibition was observed. Components tested, induced of inhibition of 44 were achieved.

photometer at a wavelength of 540. The rate of optical density decrease per minute over the linear portion of the curve was used in comparing the relative activity of the various preparations. The proportionality of the rate to enzyme concentration was established in control experiments.

Optical densities of bacterial cultures were measured on a Klett-Summerson colorimeter with a no. 54 filter. All cultures were aerated on a reciprocal shaker delivering 100 (1.5-inch) strokes per min. Temperatures were controlled in water baths to ±0.1 C.

RESULTS

Effect of NaCl on lysis and lysis inhibition. Lysis inhibition of induced K-12(λ112) was originally observed in LB medium at 44 C, whereas lysis occurred at 37 C in the same medium (Groman and Suzuki, 1962). To study the effect of LB medium on lysis and lysis inhibition, individual constituents were omitted, and the optical density of induced K-12(λ112) was observed. Of all components tested, the omission of NaCl or glucose proved to be of primary interest (Fig. 1). At 37 C, NaCl was required for lysis whereas at 44 C its presence resulted in lysis inhibition. Omission of glucose also enhanced lysis at 37 C; however, the dominance of the NaCl effect is indicated by the maintenance of lysis inhibition at 44 C even when glucose was omitted. Lysis inhibition was also observed at 44 C when K⁺, Li⁺, or Mg⁺⁺ was substituted for Na⁺ in the LB medium, although a slight decrease in optical density was observed with Li⁺ 2 to 2.5 hr after induction.

To determine whether the NaCl effects were phage-induced and specific for the mutant phage, two control series were run. The effect of NaCl omission on induced K-12(λ) is given in Fig. 2.

FIG. 1. Effect of NaCl or glucose omission on lysis of induced K-12(λ112). Cells were diluted into the appropriate medium immediately after induction and were incubated for a minimum of 4 hr in the case of lysis inhibition or until maximal lysis was achieved.

FIG. 2. Effect of NaCl omission on lysis of induced K-12(λ). Cells were diluted into the appropriate medium immediately after induction and were incubated until maximal lysis was achieved.

FIG. 3. Effect of NaCl omission on irradiated K-12. Cells were grown and irradiated in the same manner as for induction of lysogenic strains, diluted into the appropriate medium, and incubated until optical density was stabilized.
and on K-12 irradiated under the same conditions as for induction, in Fig. 3. Induced K-12(λ) lyses both in the presence and absence of NaCl at 37 and 44 °C. Thus, lysis inhibition is phage-specific and in a general sense phage-induced. It could be argued from Fig. 3 that lysis inhibition merely reflects the state of events which follows ultraviolet irradiation. However, it is already known (Groman and Suzuki, 1963) that phage and endolysin are synthesized after induction of K-12(λ112) under conditions of lysis inhibition. Moreover (Fig. 1), the increase in optical density after induction of K-12(λ112) is considerably less than that following comparable irradiation of K-12 (Fig. 3). These differences indicate that λ112 is responsible for the early cessation of growth and, by inference, for lysis inhibition.

Relationship of NaCl to lysis and lysis inhibition. In a shift of conditions from lysis inhibition to lysis, it was observed (Fig. 4) that NaCl restored some degree of lysis when added up to 150 min after induction. The sharp rise and then drop in optical density after salt addition indicates a rapid adjustment to osmotic changes. In general, the later the salt was added the shorter was the elapsed time to the initiation of lysis. This suggests that the process leading to lysis was proceeding in the absence of salt, but that some late step was dependent on its presence. It is also apparent that the percentage decrease in optical density was larger the earlier the salt was added. These observations which suggest both an early and late NaCl effect on lysis are compatible with the assumption that growth in salt for a certain amount of time is a prerequisite for the late lytic step.

That the effect of NaCl on induced K-12(λ112) is λ112-specific is supported by two pieces of evidence. Salt added to K-12(λ) at various times after induction did not alter the extent of the

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Fig. 4. Effect of NaCl addition on lysis-inhibited K-12(λ112). Induced K-12(λ112) was diluted into LB medium from which NaCl and glucose were omitted and was incubated at 37 °C; conditions producing lysis inhibition. At various times, 20% NaCl was added to bring the final concentration to 1%. The arrows indicate the curves for each time of addition.

Fig. 5. Effect of NaCl addition on lysing K-12 (λ112). Induced K-12(λ112) was diluted into LB medium from which NaCl and glucose were omitted and was incubated at 44 °C; conditions producing lysis. At various times, 20% NaCl was added to bring the final concentration to 1%. The arrows indicate the curves for each time of addition.

Fig. 6. Effect of NaCl addition on irradiated K-12(λ) and K-12. Irradiated cells were diluted in LB – (NaCl) and were incubated at 44 °C. At various times, 20% NaCl was added to bring the final concentration to 1%. The arrows indicate the curves for each time of addition. To maintain clarity, points were omitted.
post-induction period or the degree of lysis observed in the control without NaCl (see Fig. 2 for control curve). Furthermore, when K-12 was irradiated with a similar dose of ultraviolet light and salt was added at various times, the optical density continued to increase as shown by the 37 C curve in Fig. 3. It follows from these observations that the salt effect on λ112 is specific.

The effect of adding salt in a shift of conditions from lysis to lysis inhibition is given in Fig. 5. Lysis inhibition was imposed regardless of the time of NaCl addition and even after lysis was underway. This suggests that salt acts at a very late stage in the phage cycle. The data in Fig. 6 show that these effects were phage-specific and not simply due to the effect of salt on irradiated cells. The addition of salt to induced K-12(λ) did not prevent lysis, and considerable growth followed addition of salt to irradiated K-12. Both of these effects are in contrast to the stabilization observed with induced K-12(λ112). It is concluded that the salt effect is specific for induced K-12(λ112) and reflects the imposition of lysis inhibition on the culture.

**Effect of salt and temperature on λ and λ112 endolysin activity.** The data presented above show that salt and temperature influence the lytic process of induced K-12(λ112) but have no effect on induced K-12(λ). A simple hypothesis to explain these results is that λ112 endolysin differs from λ endolysin in its response to these variables.

The effect of various concentrations of NaCl, KCl, and LiCl on the in vitro activity of λ and λ112 endolysin was tested. The data for both endolysins with all these salts were virtually identical, and the pooled results are given in Fig. 7. Over the range of concentrations tested, these salts had a profound but equivalent inhibitory effect on both endolysins. The different sodium salts, sodium acetate, NaNO₃, and Na₂SO₄, gave similar results, suggesting that it was the cation that was active. These experiments were performed at room temperature because of the greater stability of the substrate at

**Fig. 7. Effect of monovalent ions on λ and λ112 endolysin activity.** Either NaCl, KCl, or LiCl was present in the assay system at the indicated molarity. Endolysins were produced in LB — (NaCl). The data represent the combined results of both endolysins tested against all three salts. The numbers in parentheses indicate the total number of values included in the mean which is given by the point. The range of values is shown by the range of the vertical lines. All assays were performed at room temperature.

**Fig. 8. Inactivation of λ and λ112 endolysin at 44 C.** Samples of endolysin were withdrawn at the indicated times, cooled, and assayed at 24 C by the modified procedure. The samples withdrawn from NaCl were diluted with LB — (NaCl) to a fixed dilution to equalize the inhibitory effect of any residual NaCl. The range of values for each point is indicated by the vertical lines. The data represent the mean of four determinations for λ and three for λ112.
Table 1. Effect of temperature on \(\lambda\) and \(\lambda 112\) endolysin activity*

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>(\lambda) endolysin</th>
<th>(\lambda 112) endolysin</th>
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<tr>
<td></td>
<td>24 C</td>
<td>37 C</td>
</tr>
<tr>
<td>1</td>
<td>1.2</td>
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<td>2</td>
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* The activities were determined by the modified procedure (see Materials and Methods) at both temperatures by use of endolysin preparations produced in LB – (NaCl). Results are expressed as the change in optical density times 10^-2 per minute.

that temperature. To simulate conditions of lysis and lysis inhibition more closely, the activity of both endolysins was compared at 37 C in LB and LB – (NaCl) by use of the modified assay. Again, no significant difference between them was observed. The relative activity of both enzymes was reduced approximately 90% in the presence of salt, a reduction similar to that observed at room temperature.

The thermostability of \(\lambda\) and \(\lambda 112\) endolysin was determined at 37, 44, and 55 C. Lysates produced in LB – (NaCl) were heated at the appropriate temperature for 30 min, cooled rapidly, and assayed at room temperature. Both enzymes were stable at 37 C, partially inactivated at 44 C, and completely inactivated at 55 C under these conditions. Both endolysins were also stable at 37 C for 30 min in the presence of 1% NaCl, i.e., in LB medium. In these latter experiments, the endolysins were diluted in LB – (NaCl) after heating and prior to assay to diminish the inhibitory effect of salt and thus increase the sensitivity of the assay. A comparison of the rates of inactivation of these endolysins at 44 C is given in Fig. 8. The rates for both endolysins are similar. While the data suggest that \(\lambda 112\) endolysin is slightly more heat-sensitive than \(\lambda\) endolysin in the absence of NaCl, the variability of the data permits no clear-cut conclusion. Both endolysins are far less sensitive to thermostability in the presence of salt.

A comparison of the relative activity of \(\lambda\) and \(\lambda 112\) endolysins at 24 and 37 C show (Table 1) that they are similar. The calculated Q10 over this range was 1.9 for \(\lambda\) and 1.8 for \(\lambda 112\) endolysin.

Discussion

The results presented show that some step in the lytic process of induced K-12(\(\lambda 112\)) is sensitive to alterations of salt and temperature in contrast to induced K-12(\(\lambda\)) which lyses under all test conditions. Thus, at 37 C lysis inhibition was observed when salt was absent, and at 44 C when salt was present, whereas lysis was observed under the reverse conditions. The observed effects are specific for \(\lambda 112\) phage and are related to phage activity rather than to some unrelated perturbation of metabolism resulting from ultraviolet irradiation.

The hypothesis that these variations in the lytic process are attributable to the direct effect of salt and temperature on \(\lambda 112\) endolysin was examined. A simplifying assumption underlying this examination was that the in vivo activities of the endolysins would be reflected in their in vitro responses. The present work shows that \(\lambda 112\) endolysin is not inactivated at 37 C and it was previously shown (Groman and Suzuki, 1963) that endolysin synthesis was not curtailed at this temperature under conditions of lysis inhibition. Therefore, a direct salt-temperature effect would have to be interpreted in terms of its influence on endolysin activity. Since lysis inhibition occurs in the absence of NaCl at 37 C and lysis occurs in its presence, the hypothesis predicts that \(\lambda 112\) endolysin would be inactive or exhibit much less activity under conditions of lysis inhibition. Quite the contrary occurs. At 37 C the activity of \(\lambda 112\) endolysin is greater in the absence of salt, and is in fact almost completely inhibited by the concentration of salt in which lysis occurs.

If some direct salt-temperature effect were involved, it might also be anticipated that \(\lambda 112\) endolysin would differ substantially from \(\lambda\) endolysin under those conditions producing lysis inhibition of K-12(\(\lambda 112\)). Campbell and Campbell-Campbell (1963) found that the endolysin induced by a lysis-inhibiting mutant of lambda was significantly more thermolabile than that induced by parental \(\lambda\), and invoked this difference in attempts to account for lysis inhibition. In the present study, both parental and mutant endolysin were inhibited to a similar extent over a wide range of salt concentrations, exhibited a similar relative activity when compared at 24 and 37 C, and had reasonably similar thermostabilities. Thus, \(\lambda\) and \(\lambda 112\) endolysins do not differ significantly under the very conditions in which induced K-12(\(\lambda\)) and K-12(\(\lambda 112\)) do differ. It appears, therefore, that the observations relating salt and temperature to lysis and lysis inhibition cannot be referred to their direct effect on endolysin action.

The locus of action of the salt-temperature effects cannot be specified at the present time. The timing of these effects indicates that a late stage in the lytic process is involved (see also...
Aside from endolysin action on the cell wall, which the present study indicates is not the step affected, the last step in the lytic process that can be visualized is one involving the integrity of the permeability barrier. It may be that λ112 is unable to carry out some critical step relative to the alteration of this barrier, with the result that under certain conditions the cytoplasmic membrane remains stabilized and lysis inhibition is observed. Whether a single explanation can, in fact, account for all the observations remains to be seen.

Acknowledgments

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Literature Cited


