T4-COLIPHAGE INFECTION OF ESCHERICHIA COLI WITH DEFECTIVE CELL ENVELOPES

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Received March 26, 1971

Summary

The development of T4 phage induced resistance to lysis from without and of lysis inhibition is prevented when Escherichia coli AS19 is the host. This bacterial host is also unusually sensitive to lysis from without by low numbers of phage during primary infection. Uninfected AS19 was found to be more sensitive than the wild type to detergents, lysozyme, EDTA, and osmotic shock. The increased sensitivity to these agents is suggestive of a defective non-specific permeability barrier, presumably the outer cell envelope. Since the biochemical basis of lysis inhibition or resistance to lysis from without has not been established it is of interest that AS19, apparently possessing a defective envelope, cannot demonstrate these functions when infected with phage.

Introduction

It has been suggested that the development of resistance to lysis from without in T4-infected E. coli involves changes in the cell envelope (1, 2). Additionally, it is likely that T4-induced lysis inhibition may also depend upon changes in the cell envelope (3, 4, 5). As a part of our attempts to elucidate the biochemical bases of these phenomena, we have examined growth of T4 phage on bacterial hosts suspected of having defective cell envelopes. One such organism is E. coli AS19. This mutant of E. coli B, first described by Sekiguchi and Iida (6), was isolated as a mutant which had increased sensitivity to actinomycin. They demonstrated that the sensitivity to actinomycin was due to an increased uptake of this antibiotic, suggestive of a defective permeability barrier. It has been demonstrated that treatment with ethylenediaminetetraacetate (EDTA) causes a partial release of the lipopolysaccharides (LPS) of the E. coli cell envelope and that such cells have a great increase in sensitivity to actinomycin (7). Since spheroplasts are sensitive to actinomycin at low concentrations (8, 9), it appears that the outer cell envelope constitutes a non-specific barrier to the antibiotic.

In this communication we describe experiments which further indicate that AS19 has a defective cell envelope, and that this defect precludes the development of phage specific functions such as resistance to lysis from without.

Materials and Methods

Bacteria and phage. E. coli AS19, obtained from B. Molholt, came originally from M. Sekiguchi. E. coli B, obtained from L. Astrachan, came originally from R. Herriott. T4r$^+$ was obtained from S. Benzer. Bacteriophage were assayed by the methods described by Adams (10).

Culture conditions. Overnight cultures of E. coli were diluted 1:100 into fresh tryptone broth and incubated with aeration at 37 C until cultures reached mid-log phase.

Assay of sensitivity to detergents and lysozyme. The bacteria to be tested were harvested in the exponential growth phase by centrifugation. After washing once the cells were resuspended in the media indicated in the text. Sensitivity to detergents or lysozyme was assayed by measuring the decrease in turbidity at 600 mμ in a Gilford 2000 recording spectrophotometer. Brij 58 (Atlas Chemical Industries, Wilmington, Delaware) and sodium deoxycholate (Nutritional Biochemicals Corp., Cleveland, Ohio) were dissolved in water and added to the cells at the indicated concentrations. Egg white lysozyme (Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in 0.05 M tris, pH 8.

Results

Lysozyme does not lyse wild type E. coli unless used in conjunction with EDTA. The chelating agent presumably removes surface cations, resulting in the release or distortion of cell envelope components, thereby making the mucopeptide more accessible to lysozyme. Sekiguchi and Iida (6) reported that AS19, when suspended in tris buffer, could be lysed without the addition of EDTA. This is confirmed in Fig. 1, which also indicates that AS19 can be lysed by egg white lysozyme when the cells are suspended in 1 per cent

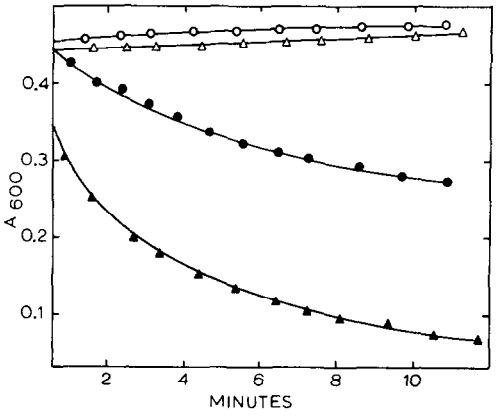

Fig. 1. Sensitivity of E. coli AS19 to lysozyme. Cell suspensions were prepared as described in Materials and Methods. At the beginning of the experiment lysozyme was added at a concentration of 2.0 µg/ml. Symbols: O, cells in tris; Δ, cells in 1 per cent tryptone; ●, cells in tris + lysozyme; ▲, cells in 1 per cent tryptone + lysozyme.

tryptone. Although not shown, when resuspended in tryptone broth (containing 0.1 M NaCl) the cells were insensitive to lysozyme.

Anionic detergents cause rapid disintegration of the cell membrane. Intact Gram negative cells, however, are quite insensitive to sodium deoxycholate (DOC). As shown in Fig. 2, AS19 is more sensitive to DOC lysis than is strain B, being lysed by 3.0 mg/ml. In other experiments it was shown that DOC at a concentration of 10 mg/ml did not lyse B. A similar increase in the sensitivity of AS19 to sodium lauryl sulfate could also be shown.

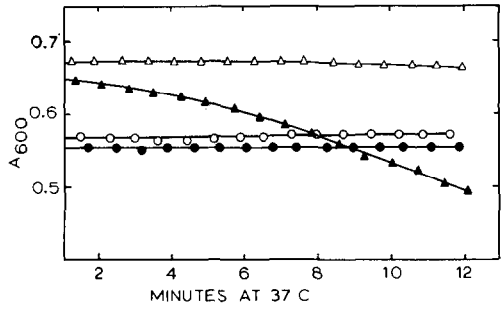

Fig. 2. Deoxycholate lysis of E. coli AS19. Cells were grown as described in Materials and Methods, and after washing resuspended in 0.05 M tris, pH 8. Symbols: Δ, AS19, no DOC; ▲, AS19 + 3 mg/ml DOC; O, B, no DOC; ●, B + 3 mg/ml DOC.

To determine the extent of the defect in the outer envelope of AS19 we examined its sensitivity to Brij 58. Brij 58 is a non-ionic detergent which can disaggregate E. coli spheroplasts, but not intact cells (11). AS19 was resistant to this detergent, indicating that sufficient outer envelope is retained to exclude the detergent. This was further indicated in the comparison of sensitivity to Brij 58 of spheroplasts of AS19 prepared either with lysozyme and EDTA, or with lysozyme alone (Fig. 3). Apparently EDTA is required to release outer cell envelope components to expose the membrane to Brij 58. The retention of at least part of the outer envelope is also indicated by the report that AS19 is sensitive to all of the T-phages which have their receptors in the cell envelope (6).

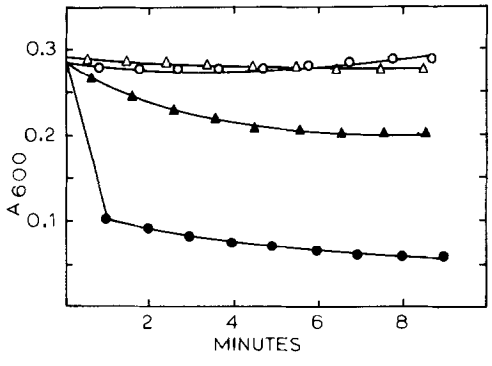

Fig. 3. Lysis of E. coli AS19 spheroplasts by Brij 58. Spheroplasts were prepared as described in Materials and Methods, using either lysozyme alone (Δ, ▲), or lysozyme + EDTA (O, ●). Addition of Brij 58 during assay, ▲, ●.

The outer cell envelope of Gram negative bacteria contains both lipoprotein (LP) and LPS (12). The preceding experiments suggested that AS19 might be defective, but not completely deficient in these structures. To further implicate LPS or LP (or both) we examined the sensitivity of AS19 to osmotic shock. It has been reported that Pseudomonas strains release LPS and LP when treated with EDTA and that such cells were increasingly sensitive to osmotic shock (13). We therefore compared the loss of viability of E. coli strains B and AS19 after osmotic shock and EDTA treatment. Table 1 indicates that AS19 is sensitive to osmotic shock without EDTA treatment.

Table 1. Viability of E. coli after EDTA and osmotic shock

	Viable		Per cent survivors	
	B	AS19	B	AS19
Saline	1.20×10^6	1.76×10^6	100	100
H_2O	1.38×10^6	1.66×10^5	115	9.4
EDTA	6.70×10^5	4.60×10^4	56	2.6

Log phase cells were diluted 100-fold into the indicated media. After incubation at 37 C for 30 minutes survivors were assayed by plate assay. Per cent survivors was calculated by dividing survivors in indicated media by those after saline incubation. EDTA was used at concentration of 0.001 M.

This may also explain why AS19 is lysed by lysozyme when suspended in 1 per cent tryptone, but not when in tryptone broth containing 0.1 M NaCl.

The biochemical basis of lysis inhibition or resistance to lysis from without has not been established. It has been suggested (1, 2) and it would be reasonable to expect that resistance to lysis from without by superinfecting phage should require changes in the cell envelope of the host cell.

Table 2. Resistance to lysis from without

Bacteria	Fraction absorbance after superinfection	Fraction infective centers after superinfection
E. coli B	0.87	1.0
E. coli AS19	0.40	0.45

Log phase bacteria were infected with 6 T4 phage per bacterium at 37 C. At 6 minutes after infection they were superinfected at a multiplicity of 200. Infectious centers were measured by diluting the cells into phage antiserum (k = 10) at 13 minutes and then plating after 3 minutes of further incubation. Fraction absorbance and fraction infective centers were calculated by dividing the values for the superinfected samples by values obtained for non-superinfected but otherwise identical samples.

Table 2 indicates that AS19 cannot become resistant to lysis from without when infected with T4 wild type phage. This was of interest because this function has been attributed to the s (spackle) gene of T4 phage (2).

The s gene of T4 is well separated from the r gene (lysis inhibition) in T4 (2). Nevertheless, T4Bs mutants, in addition to being unable to confer resistance to lysis from without, also have lost the ability to demonstrate lysis inhibition. Since AS19 cannot demonstrate resistance to lysis from without we attempted to determine if it could show lysis inhibition when infected with T4r^+. Wild type phage, when plated on AS19 were found to consistently form plaques with r-phenotypes, indicative of loss of lysis inhibition. One step growth experiments were usually unsuccessful, resulting in low recovery of infectious centers. This was especially true when cells were superinfected at low multiplicities to enhance the chances for lysis inhibition. Subsequently it was found that AS19 is abnormally sensitive to lysis from without, even at low multiplicities of primary infection (Table 3). Since AS19 is incapable of developing resistance to lysis from without after primary infection, the loss of infectious centers in one step growth

Table 3. Sensitivity to lysis from without by T4

Input multiplicity	Infected OD_{600}/Control OD_{600}	
	E. coli B	E. coli AS19
5	1.03	0.94
10	1.03	0.89
20	1.01	0.70
30	1.05	0.59
40	1.00	0.28

Log phase cells were infected with T4 at the indicated input multiplicities. After 5 minutes incubation at 37 C the decrease in turbidity at 600 mμ was measured.

experiments presumably occurred as a consequence of extensive lysis upon low secondary multiplicities of superinfection.

Discussion

E. coli AS19 was selected for its sensitivity to actinomycin D (6). The data presented here demonstrate that this organism is also sensitive to EDTA, osmotic shock, ionic detergents, and that it is defective in certain aspects of phage replication. Since spheroplasts of E. coli are sensitive to actinomycin D and to detergents, but wild type intact organisms are not, it appears that the outermost layers of the cell envelope constitute a non-specific barrier to these agents. The enhanced sensitivity of AS19 to these agents would therefore imply an alteration or a defect in this barrier. It is clear, however, that this non-specific barrier is not completely missing since phage receptor sites are retained and spheroplasts prepared without use of EDTA are not completely sensitive to nonionic detergents.

It is easy to understand the usefulness of a non-specific permeability barrier. It was of great interest however to find that such a barrier may also play a role in certain phage functions. For example, it has long been known that E. coli can be lysed from without by high multiplicities of phage during primary infection (1) and that phage bound lysozyme is not required (3). If wild type phage are used at a low multiplicity the host cells become resistant to lysis from without within a few minutes after primary infection. This process certainly could be expected to require either synthesis or modification of cell envelope components. The data presented here indicate that the defect in the envelope of AS19 is such that it precludes alteration by phage gene specific products which may be required for lysis inhibition and/or resistance to lysis from without.

Experiments to more precisely define the biochemical nature of the defect in the cell envelope of AS19 are in progress. We anticipate that characterization of the defect will aid in understanding the role of the cell envelope in phage infection.

Acknowledgments

This investigation was supported by Public Health Service research grant AI 06875 from the National Institute of Allergy and Infectious Diseases.

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