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Lysis of *Escherichia coli* cells induced by bacteriophage T4

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Abstract: Structural changes in the envelope of *Escherichia coli* cells accompanying their lysis from without by bacteriophage T4 have been studied. The hypothesis concerning the role of collapse of membrane potential and formation of periplasmic vesicles in the process of lysis from without has been advanced.

Key words: Phage infection; Multiplicity of infection; Membrane potential; Membrane invagination; Periplasmic vesicles; *Escherichia coli*

Introduction

Bacteriophage T4 is known to be capable of lysing cells of *Escherichia coli* by two different processes [1–3]. Firstly, lysis of cells, as a necessary stage of the living cycle of phage, is observed before the newly formed phage particles leave the cytoplasm at later stages of infection (lysis from within). Secondly, the phage interacting with the outer surface of the bacterial cell can induce lysis upon a great multiplicity of infection (so-called lysis from without).

Up to now the mechanism of both processes has been studied rather superficially [3]. It is commonly accepted that bacteriophage lysis from within is the result of the accumulation of phage-encoded lysozyme during late protein synthesis. Despite some available evidence on direct participation of phage protein (e lysozyme) in lysis from within [4,5], it was subsequently found that some mutants, which are deficient in the product of gene e, are also able to initiate lysis [2,6]. The process of lysis was shown to be capable of being induced by the product of gene 5, i.e. by a protein belonging to the central part of the T4 baseplate [6].

The process of lysis from without complicates our thinking about T4 lysis. It is known that mutations in gene 5 abolish the ability to cause lysis from without [6]. It was suggested that gp5 is responsible for the lytic activity which is intrinsic to the virion and probably acts to cause localized degradation of the murein layer during the infection process [3]. However, the mechanism of lysis from without and the structural changes in the cell membranes taking place during lysis have practically not been studied.

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It was shown that in the course of T4 infection a fusion of the cell membranes and formation of intermembrane bridges occurred [7,8]. Besides, in the case of a great multiplicity of infection, vesicles in the periplasmic space are formed as a result of abortive infection of cells by some phage particles [8]. Diverse structural changes accompanying the lysis of the infected cells could be found as well [8]. In the present work we describe structural changes in the membranes observed in the course of lysis from without and suggest a hypothetical scheme permitting the description of the observed structural changes as a chain of events beginning from interaction of the phage particles with the cellular surface and ending with a disturbance of integrity of the cellular envelope and release of the cytoplasmic contents into the environment.

Materials and Methods

E. coli cells were grown in tryptone broth and harvested in the middle of the logarithmic phase of growth. Before infection the cells were placed into fresh broth (6×10^8 cells/ml). Phage T4D was grown and purified according to the method described previously [9]. Multiplicity of infection was about 200 phage particles per bacterial cell. Lysis of the cells was monitored by changes in the optical density (650 nm) of the suspension. Ultrarapid plunge-freezing without cryoprotectants with subsequent fracturing or ultrathin sectioning followed by chemical fixation were described before [7]. For ultrathin sections, the cells were sequentially fixed with 1% solutions in 100 mM phosphate buffer (pH 7.4) of glutaraldehyde, osmium tetroxide, tannic acid and with osmium tetroxide again (GOTO-fixation; [7]). After every fixation step the samples were washed three times in phosphate buffer.

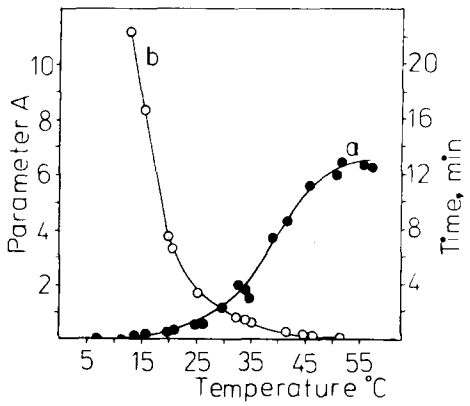

Fig. 1. Temperature dependence of lysis from without of *Escherichia coli* cells infected by bacteriophage T4. The cells were infected by T4 phage at an infection multiplicity of 200. Parameter A (curve a) is the initial rate of lysis from without induced by T4 phage. The initial rate lysis from without was calculated from the linear part of the curve of change in optical density of cell suspension induced by T4 infection. Curve b characterizes the time delay (min) of beginning of lysis from without.

Results and Discussion

Decreasing the temperature decreased the rate of lysis from without induced by the phage and increased the time requred to initiate lysis (Fig. 1). At temperatures above 45°C, lysis began just after phage had been added to the cell suspension. Below 14°C, there was no significant lysis. This is also the temperature at which the potassium efflux induced by T4 phage stops [10]. The lipid hydrocarbon chains of *E. coli* display a relatively broad order–disorder transition extended over 20°C and with a mid-temperature of transition between 16 and 18°C [10]. In connection with this we think that the ordering of the lipids may prevent lysis from without.

When the *E. coli* cells were infected with a great multiplicity of phage T4 (above 100 particles per cell), one of the most obvious structural changes in the cell membranes was the formation of large vesicles in the periplasmic space (Fig. 2A). Upon a low multiplicity of infection (5–10) of the cells treated with CCCP, the large vesicles are also forming [7,8]. These vesicles are formed by invagination of the outer membrane under the action of some phage particles on the cellular surface. We have assumed that formation of such structures is due to the process of abortive infection. As seen in Fig. 2A, periplasmic vesicles are connected with the outer membrane, which con-

firms our hypothesis about participation of invagination of the outer membrane of bacterial cell in the formation of vesicles. The membrane vesicles closely interact with the inner membrane [7,8], which is accompanied by the appearance on the fracture surface of round regions, deficient in intramembrane particles (Fig. 2B).

Infection of cells for 5–10 min is accompanied by structural changes in the cellular envelope, which are interpreted by us as part of a process of cellular lysis. These changes are characterized by formation of bulges of the membrane in some local parts of the bacterial cell that leads to the swelling and pinching out of the vesicles (Fig. 3A). Observations show the pinching out of the vesicles is accompanied with a fusion of outer and inner membranes of the bacterial cell (Fig. 3B, B'). As a result, hole structures, through which the cytoplasmic contents are released, are formed (Fig. 3C). At the final stage we usually observe empty (without any cytoplasmic content) 'shadows' of *E. coli* cells where holes are seen (Fig. 3D). In all micrographs presented in Fig. 3, the holes are formed by fusion between the outer and inner membranes.

We believe that there is a relationship between formation of periplasmic vesicles observed at early stages of infection and later processes of cellular lysis. Indeed, the invagination of the outer membrane and formation of periplasmic vesicles results in development of local defects in the cell envelope capable of decreasing its resistance to the osmotic pressure of cytoplasm. In accord with our hypothesis, the integrity of the peptidoglycan layer is disrupted at the sites of formation of periplasmic vesicles (Fig. 4A,B). We do not deny the possibility that peptidoglycan rupture may occur with the participation of *E. coli* enzymes ('host' autolysis) activated in the course of collapse of membrane potential and invagination of the outer membrane. These membrane sites could be 'weak points' of the cellular envelope where

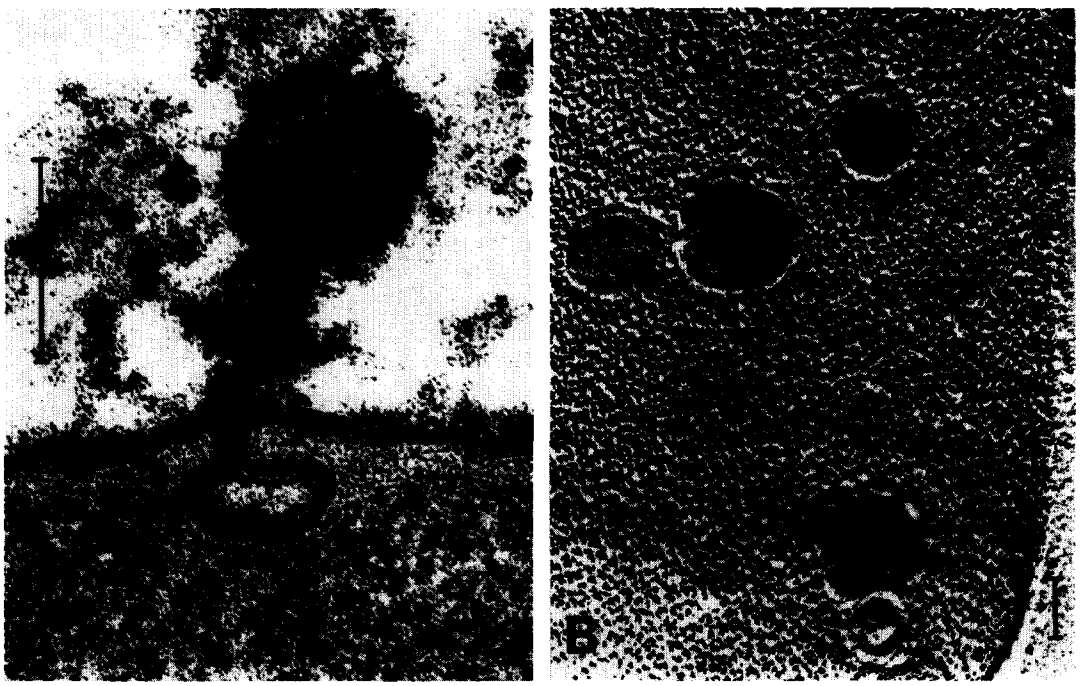

Fig. 2. Ultrathin sections (**A**) and freeze-fracture replicas (**B**) of *Escherichia coli* infected by phage T4 at 20°C for 5 min. Phage were added at a multiplicity of 200. The invagination of the outer membrane and formation of periplasmic vesicles induced by some phage particles (**A**) are accompanied by structural defects (round smooth areas) on the fracture surface of the inner membrane (**B**). Bars represent 0.1 mm.

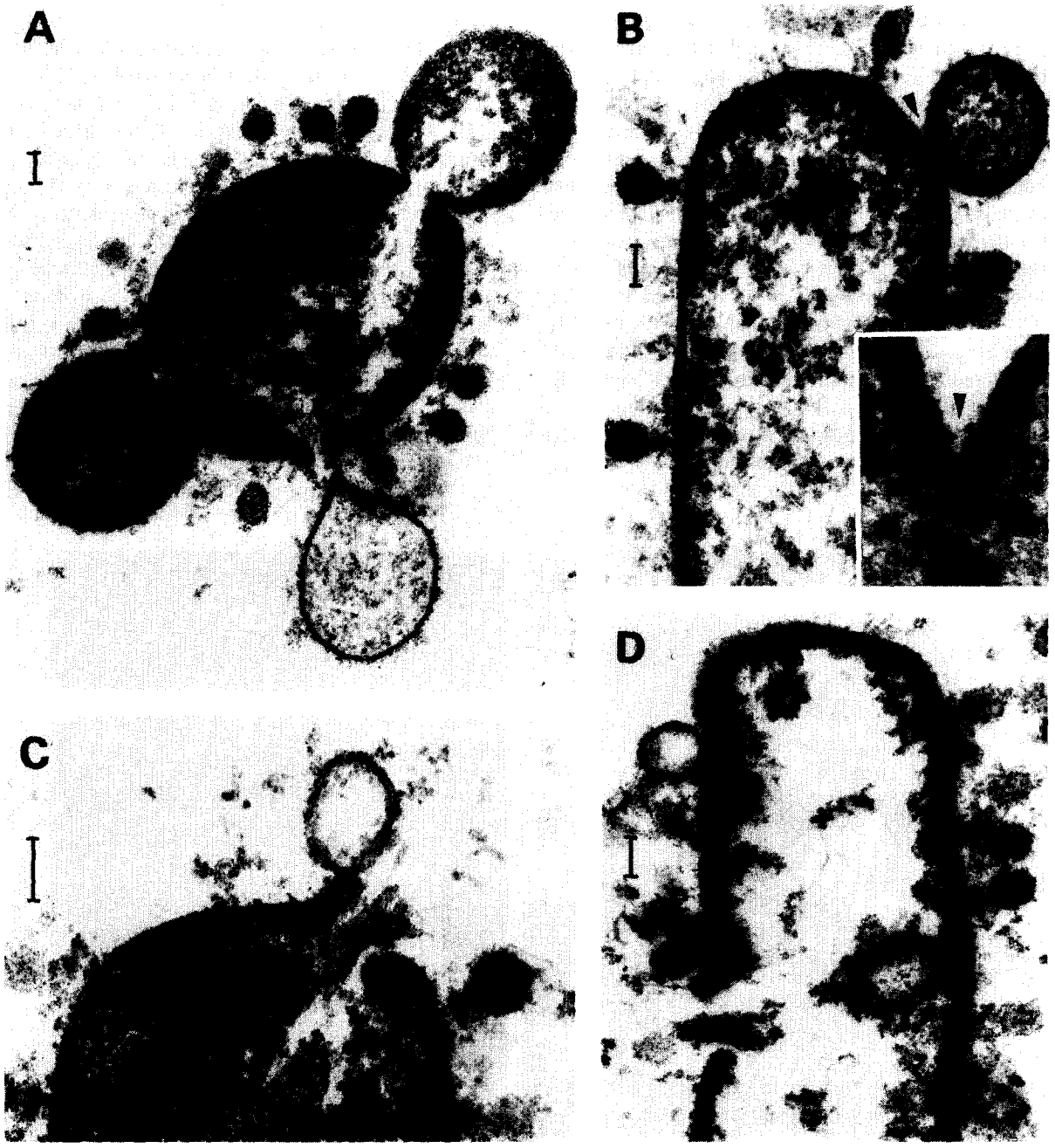

Fig. 3. Structural changes in the membranes of *Escherichia coli* cells infected by phage for 10 min. The swelling and pinching out of the membrane vesicles are rather typical for those cells (**A**). The pinching out of the membrane vesicles (**B**) is accompanied by fusion of the outer and inner membranes (**B'**, arrowheads). As a result, the hole structures are formed (**C**) and contents of cytoplasm are released (**D**). Bars represent 0.1 mm. Multiplicity of infection was about 200.

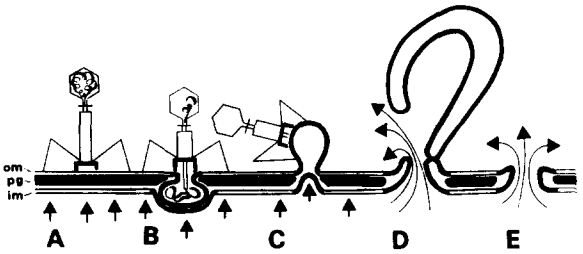

Fig. 4. Schematic representation of sequential stages of lysis from without of *Escherichia coli* cells initiated by phage T4.

the swelling processes are initiated by osmotic forces (Fig. 4C). In line with the presented scheme, the release of vesicles from the cell surface is accompanied by fusion of the neighboring membranes (Fig. 4D) and formation of the hole structure (Fig. 4E). We suggest that in the case of a great multiplicity of infection (above 100 phages per cell) the collapse of membrane potential and formation of vesicles in the periplasmic space induced by the phage particles are the important factors determining the lytic activity of T4 bacteriophage in the process of lysis from without. These results support the ideas that the implementation and regulation of lysis involve important phenomena such as protein–membrane interactions and membrane energetics [3].

Acknowledgements

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