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Outer Membrane Porins Are Important in Maintenance of the Surface Structure of *Escherichia coli* Cells

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Escherichia coli cells lacking the OmpF and OmpC proteins, porin proteins of the outer membrane, are often unstable and easily revert to strains which either have regained one or both of these proteins or contain a new outer membrane protein. The structural importance of porin proteins in the cell surface was studied in the present work. Tris-hydrochloride buffer at a concentration of 120 mM caused deformation of the cell surface of a strain lacking these porins; the undulated appearance of the negatively stained cell surface changed to a smooth and expanded form. The Tris-induced deformation was seldom observed with either the wild-type strain or a pseudorevertant that possessed the OmpF protein. The role of the OmpF protein in stabilizing the cell surface against Tris treatment could be slightly taken over by the LamB protein, which shares a number of unique properties with the former proteins. The deformation of the cell surface by Tris-hydrochloride buffer was accompanied by a loss of viability, the lethal damage being especially significant when the cells lacked porins. Upon induction with maltose, cells with the undulated appearance could absorb lambda phages, whereas the deformed cells could not. These results suggest that the instability of cells lacking porins is primarily due to a structural defect of the outer membrane.

OmpF and OmpC porins are major proteins of the outer membrane of *Escherichia coli* K-12 and share a number of biochemical and physicochemical characteristics. These include a firm association with the peptidoglycan layer (5, 11, 23), an extremely tight trimer structure that is resistant to sodium dodecyl sulfate (17, 18, 31), a high content of β-structure (18, 23), and formation of pores for small hydrophilic molecules (19). Although the OmpF and OmpC proteins are dispensable, they are apparently important for cell survival. The *ompB* mutants which lack both OmpF and OmpC proteins are rather unstable and frequently produce either pseudorevertants with one or both of the proteins (24) or proteins functionally similar to the OmpF and OmpC proteins (6, 12, 22). This instability has been discussed in relation to defects in pore function (12, 22, 24). Indeed, outer membrane permeability and usage by the cells of some sugars, amino acids, and ions are decreased in *ompB* mutants (12, 21, 26). The importance of these proteins from the structural point of view has also been suggested. They are called matrix proteins since they are observed as a hexagonally latticed array that covers the entire surface of the cell envelope when the envelope is treated with sodium dodecyl sulfate (23). Such a hexagonal array can be reconstituted on the peptidoglycan layer from these proteins and lipopolysaccharide, indicating that these proteins are major constituents of the basal framework of the cell surface (14, 28, 29).

Here we report additional evidence from in vivo experiments that the porin proteins are structurally important. We found that loss of the OmpF and OmpC proteins caused by the *ompB* mutation made the cells susceptible to Tris-hydrochloride regarding both surface structure and viability. This effect of Tris was not significant in either the *ompB*$^+$ parent strain or a pseudorevertant that produces the OmpF protein.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. *E. coli* AB2847 (K-12 F$^-$ *aroB tsx malT supE* $\lambda^-\lambda^r$) (1) and its isogenic *ompB* strain, T19 (*tsx ompB supE*) (15), were kindly donated by T. Nakae. Strain T19R4, isolated in this study, is a spontaneous mutant of T19 that produces the OmpF protein in the *ompB* background. Phage lambda was heat-induced from *E. coli* CSH45 [K-12, *lac thi trpR* (λ *cI857 S7*)] (13) and purified on a CsCl gradient.

Bacterial cultivation and treatment with Tris-hydrochloride. Bacteria were grown in 5 ml of tryptone broth (10 g of tryptone [Difco] and 2.5 g of NaCl per liter) at 37°C with shaking. For induction of the LamB protein, the receptor for the lambda phage, the medium was supplemented with maltose (4 g/liter). Early-log-phase cells (3×10^8/ml) were collected by centrifugation (3,000 rpm for 10 min) from the 5 ml-culture, washed

with 2 ml of 120 mM Tris-hydrochloride (pH 8.0), and suspended in 2 ml of the same buffer. These operations were quickly carried out at room temperature (ca. 20°C). The cell suspensions thus prepared were immediately incubated at 37°C, and morphological changes of the cell surface were examined under a Hitachi HS-9 electron microscope after negative staining with 1% phosphotungstate (pH 6.2). For viability measurements, cells grown in tryptone broth were directly diluted with the Tris buffer to a density of about 5×10^3/ml and incubated at 37°C. A sample (0.1 ml) of the suspension was plated on tryptone agar, and colonies that appeared after overnight incubation at 37°C were counted.

RESULTS

Effect of Tris-hydrochloride on cell surface appearance. In the course of studying the process of infection by the lambda phage of *E. coli* T19, we noticed that 120 mM Tris-hydrochloride inhibited adsorption of the phage to the cell surface. This prompted us to examine the effect of Tris-hydrochloride on the cell surface. Figure 1A is an electron micrograph of negatively stained strain T19 grown in tryptone broth. The entire cell surface has an undulated appearance. This is the general profile of many negatively stained gram-negative bacteria and most likely is that of the outer membrane (2). Treatment with 120 mM Tris-hydrochloride at 37°C caused significant changes on the cell surface; namely, the cell surface expanded and became smooth, and undulation was no longer observed (Fig. 1B). The deformed cells were 1.1 to 1.2 times wider than they were before the Tris treatment (see also Fig. 2 and 4). Three layers, possibly corresponding to the outer membrane, peptidoglycan layer, and cytoplasmic membrane, were observed separately. The high concentration of Tris was found to be responsible for this effect, for incubation with 10 mM Tris-hydrochloride at the same pH did not result in such morphological changes (data not shown).

Porins and cell surface stability. It was suggested that lack of the OmpF and OmpC proteins is responsible for the effect of Tris, since strain T19 lacks these proteins owing to the *ompB* mutation and since many *E. coli* strains that are wild-type for these outer membrane proteins were found to be resistant to the Tris treatment. To confirm this theory, we tested the *ompB*$^+$ parent strain of strain T19 (AB2847) and an OmpF-possessing pseudorevertant of strain T19 (T19R4) for Tris sensitivity (Fig. 2; Table 1). Although strain AB2847 is genetically *ompC*$^+$, the amount of the OmpC protein was almost nil under the cultivation conditions employed here. It was noticed that the morphological changes occurred almost unequivocally in individual cells; i.e., an intermediate profile was hardly observed. Therefore, the fraction of cells that had been deformed could be estimated rather easily (Fig. 2F). More than 80% of the T19 cells were converted to the expanded form during 30 min of incubation in 120 mM Tris-hydrochloride, whereas strains AB2847 and T19R4 were appreciably resistant to the Tris treatment, indicating that the OmpF protein is responsible for maintaining the stable structure of the outer membrane.

The LamB protein shares a number of unique properties with the OmpF and OmpC proteins. These include an extremely tight trimer structure that is resistant to denaturation by sodium dodecyl sulfate (19, 20), a characteristic association with the peptidoglycan layer (4), the ability to assemble into a hexagonal lattice that covers the entire surface of the peptidoglycan layer (30), and formation of pores for small hydrophilic molecules (3, 7, 10, 15, 16). Therefore, the possibility was investigated that the LamB protein could replace the OmpF protein in terms of resistance to the Tris treatment. Induction with maltose of the LamB protein resulted in an increase to a certain extent of resistance to the Tris treatment (Table 1). However, the cells were still appreciably sensitive to the Tris treatment in comparison with cells (strains AB2847 and T19R4) that possess the OmpF protein. Gel electrophoretic analyses of the envelope proteins showed that the amount of the LamB protein in the maltose-induced T19 cells was roughly the same as that of the OmpF protein in AB2847 and T19R4 cells (data not shown), eliminating the possibility that the weakness of the LamB protein-containing cells is due to the quantity of the LamB protein. It should also be noted that even though they still possessed the OmpF protein, T19R4 cells became more sensitive to the Tris treatment upon induction of the LamB protein. These results, taken together, suggest that although the role of the OmpF protein can be partly taken over by the LamB protein, the presence of the latter is insufficient to make the outer membrane highly resistant to the Tris treatment.

Porins and cell stability. The OmpF protein protected the cells from the lethal damage caused by the Tris treatment (Fig. 3). Strains T19R4 and AB2847 were appreciably resistant to the Tris treatment in terms of viability, whereas the viability of strain T19 decreased quite rapidly in 120 mM Tris-hydrochloride at 37°C. A lower concentration of Tris-hydrochloride (10 mM) did not result in serious damage to the cells. Thus, the decrease of viability was closely related to the deformation of the cell surface described above, most likely suggesting that the deformation causes cell death.

Effect of Tris treatment on lambda phage adsorption to the cell surface. As stated above, 120

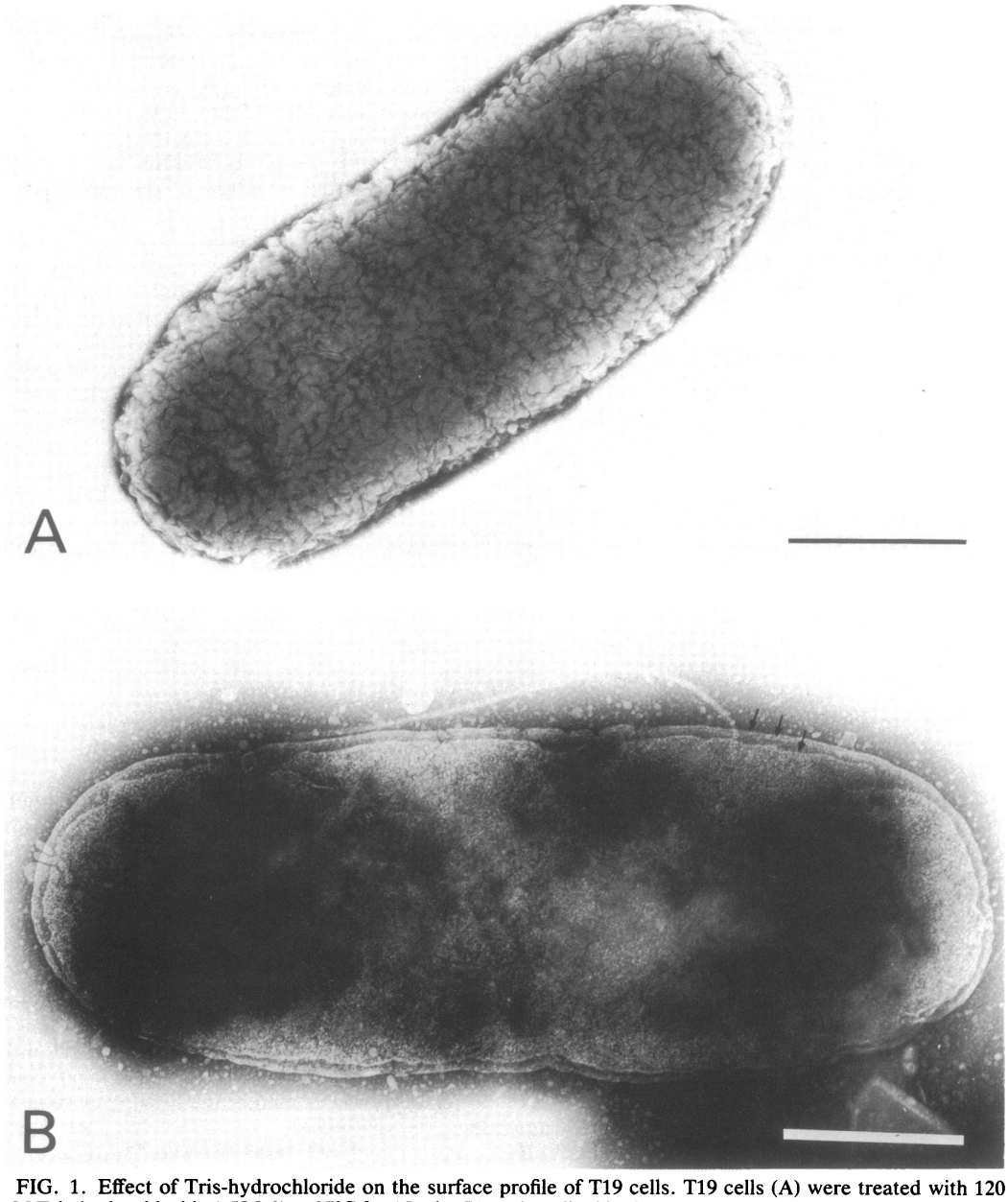

FIG. 1. Effect of Tris-hydrochloride on the surface profile of T19 cells. T19 cells (A) were treated with 120 mM Tris-hydrochloride (pH 8.0) at 37°C for 15 min (B) as described in the text, were negatively stained, and then were examined under an electron microscope. The bar represents 500 nm. Arrows possibly indicate (starting from the outside) the outer membrane, peptidoglycan layer, and cytoplasmic membrane.

mM Tris-hydrochloride inhibited adsorption of the phage to the cell surface. Here we examined whether the inhibition was due to the presence of 120 mM Tris or was a result of the Tris-induced deformation of the cell surface. Strains T19 and T19R4 were cultured in the presence of maltose to induce the LamB protein and were treated with 120 mM Tris. Then adsorption of the lambda phage to the cells was examined under an electron microscope. In both strains, cells showing undulation adsorbed a large number of phage particles, whereas the deformed cells were unable to do so. Typical examples are shown in Fig. 4, indicating that the lack of phage

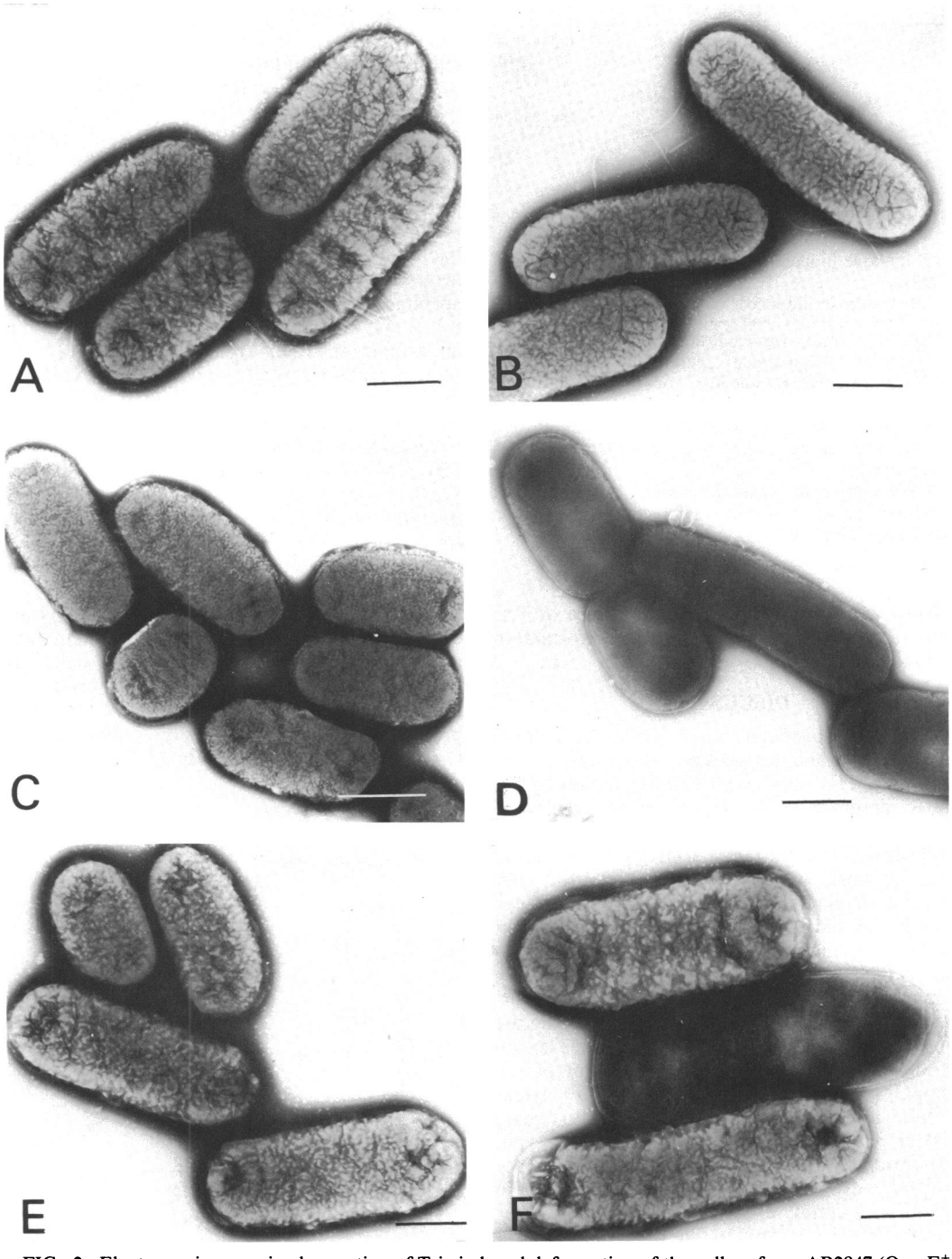

FIG. 2. Electron microscopic observation of Tris-induced deformation of the cell surface. AB2847 ($OmpF^+$) (A and B), T19 ($OmpF^-$) (C and D), and T19R4 ($OmpF^+$) (E and F) cells were examined under an electron microscope before (A, C, and E) and after (B, D, and F) incubation with 120 mM Tris-hydrochloride (pH 8.0). The procedures were the same as those described in the legend to Fig. 1. The bars represent 500 nm.

TABLE 1. Effect of the OmpF and LamB proteins in the outer membrane on Tris-induced deformation of the cell surface[a]

Strain	Relevant proteins present[b]	Fraction of deformed cells (%) after incubation for:	
		15 min	30 min
AB2847	OmpF	15	20
T19		76	83
T19 (maltose induced)	LamB	53	83
T19R4	OmpF	22	21
T19R4 (maltose induced)	OmpF LamB	30	30

[a] *E. coli* strains were cultured in the absence and presence of 0.4% maltose, treated with 120 mM Tris-hydrochloride (pH 8.0) at 37°C for the indicated periods, and examined under an electron microscope. About 500 to 1000 cells were examined at random, and the fraction of cells that had been deformed was determined.

[b] Although strain AB2847 is *ompC*$^+$, the amount of the OmpC protein was almost nil under the cultivation conditions employed.

adsorption was not due to the presence of Tris but was a result of the cell surface deformation induced by Tris.

DISCUSSION

Tris buffer has been shown to interact with the outer membrane to weaken its structure. For example, Tris acts as an organic cation to the outer membrane (25, 27), causes the release of outer membrane components (8, 25), and increases outer membrane permeability (9). In the present work, we found that Tris caused substantial changes in the morphology of the outer membrane and resulted in cell death. The effect of Tris became extraordinarily significant when the cells lacked the outer membrane protein OmpF, indicating that the protein was important, although not indispensable, for keeping the outer membrane structure stable. Since strain AB2847 contains a very small amount of the OmpC protein in the *ompC*$^+$ *ompB*$^+$ background and since we failed to isolate isogenic strains with the OmpF$^-$ OmpC$^+$ phenotype, experiments with the OmpC protein were not carried out. However, considering that the OmpF and OmpC proteins resemble each other extensively, it is assumed that the OmpC protein behaves in a manner similar to the OmpF protein in this respect as well.

The LamB protein shares a number of unique properties with the OmpF and OmpC proteins, and indeed it stabilized outer membranes that lack the OmpF and OmpC proteins against the Tris treatment to a certain extent. However, outer membranes that have the LamB protein in place of the OmpF and OmpC proteins were not so stable in terms of the Tris resistance. Furthermore, introduction of the LamB protein into outer membranes which contained the OmpF protein weakened their resistance to Tris. It is concluded, therefore, that the OmpF protein cannot be fully substituted for by the LamB protein in terms of Tris resistance.

The undulated appearance of the cell surface is typical of negatively stained gram-negative bacterial cells (Fig. 1A). On the other hand, thin-sectioned specimens, as well as freeze-etched specimens, usually have no undulated profiles (2). It may be, therefore, that such undulation is an artifact resulting from cell drying (2). Although we do not yet have conclusive evidence, in the present work the undulated appearance was observed when the cell surface was thought to retain a stable structure, i.e., in the absence of 120 mM Tris or in the presence of porins. Furthermore, the fact that the lambda phage distinguishes the undulated cells from the deformed cells (Fig. 4) indicates that their respective surface structures were different even before drying.

Although the OmpF and OmpC proteins are dispensable, cells which lack both of them have a growth disadvantage. When such cells are cultured for a long period, mutants producing either one or both of the proteins (24) or a protein which is functionally similar to the OmpF and OmpC proteins (6, 12, 22) frequently

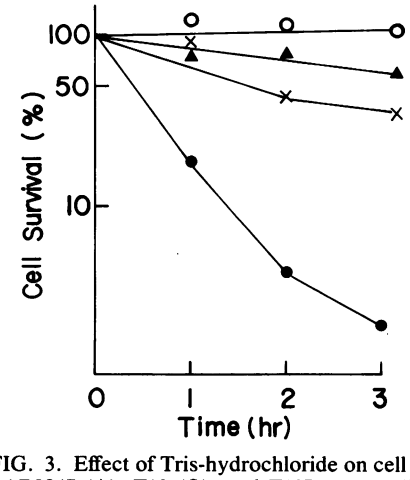

FIG. 3. Effect of Tris-hydrochloride on cell viability. AB2847 (▲), T19 (●), and T19R4 (×) cells were incubated with 120 mM Tris-hydrochloride (pH 8.0) at 37°C for the indicated periods, and cell viability was measured as described in the text. As a control experiment, T19 cells were incubated in 10 mM Tris-hydrochloride (pH 8.0) (○).

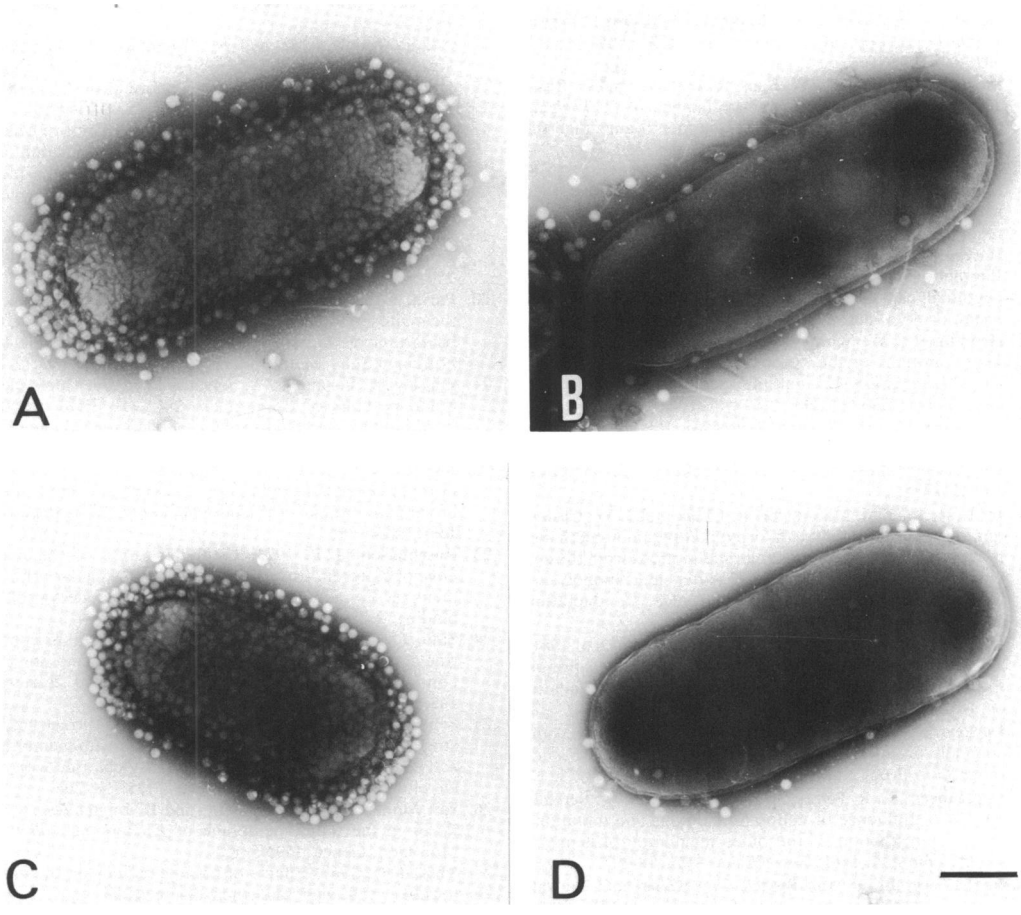

FIG. 4. Adsorption of lambda phage to T19 and T19R4 cells treated with 120 mM Tris-hydrochloride. *E. coli* T19 (A and B) and T19R4 (C and D) were incubated with 120 mM Tris-hydrochloride (pH 8.0) for 15 min. After addition of magnesium sulfate to a final concentration of 0.5 mM, cells were infected with lambda (multiplicity of infection, 100) and further incubated for 5 min at 37°C. Samples were negatively stained and examined under an electron microscope. Typical profiles of undulated (A and C) and deformed (B and D) cells in Tris-treated samples are shown. The bar represents 500 nm.

accumulate. Since these proteins have pore functions, the advantages of possessing them have been mainly discussed in relation to efficient uptake of nutrients through the pores (12, 22, 24). In contrast, the present work shows the structural importance of these porins for making the outer membrane stable. This is consistent with a previous reconstitution study which showed that the OmpF and OmpC proteins could be assembled with lipopolysaccharide to form a stable hexagonal lattice structure which could cover the entire surface of a peptidoglycan layer (14, 28, 29). The structural importance of these proteins is also suggested by the fact that a lack of these proteins makes the cells sensitive to sodium dodecyl sulfate (12). The present work further shows that the unstable viability in Tris buffer of cells lacking the OmpF and OmpC proteins is not due to a defect in the pore function. Although the experimental conditions employed here were not physiological (120 mM Tris buffer), these results suggest that the instability of the cells lacking these proteins is primarily due to a structural defect rather than a defect in pore function of the outer membrane.

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