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EXPERIMENTAL ARTICLES

The Effect of Composition of the Core Region of *Escherichia coli* K-12 Lipopolysaccharides on the Surface Properties of Cells

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Abstract—The pH dependences of electrokinetic potentials (EKP) of the cells of two *Escherichia coli* K-12 strains (D21 and JM 103) with known lipopolysaccharide (LPS) core composition have been determined by the method of microelectrophoresis. At pH 4.6–5.2, the negative surface charge of the cells with Re core LPS was reliably higher. It was shown that the interaction of bacteria with lysozyme results in a decrease of optical density of suspensions due to higher sensitivity of the cells with complete LPS core to hypotonic shock. LPS release from bacterial cell wall depended also on bacterial LPS core composition and increased with LPS core extension. Electrokinetic measurements and the study of the interaction of cells with lysozyme suggest that higher negative surface charge of *E. coli* JM 103 cells (Re type LPS) is associated with higher quantity and density of LPS packing in the cell wall as compared with the cells of *E. coli* D21 (Ra type LPS).

Key words: Escherichia coli, lipopolysaccharide, core composition, electrokinetic properties.

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The cell wall of gram-negative bacteria is a highly organized structure providing structural rigidity and cell survival. The surface of gram-negative bacteria contains LPS as the major component of the outer leaflet of the outer membrane, participating in the interrelation between bacterial cells and the environment [1]. LPS molecules consist of a hydrophobic lipid A, hydrophilic inner and outer core, and repeating units of O-antigen [2]. Approximately 3.5 million LPS molecules are present on the surface of a gram-negative bacterial cell [3], but the nature of the LPS packing on bacterial surface is presently not fully known.

It is known that LPS in the outer membrane form regions of 600–3500 densely packed molecules [4]. These LPS regions act as a permeability barrier for many small molecules, including some antibiotics. Besides LPS, the outer membrane contains certain proteins, in particular, porins, which form channels for the molecules below 700–1000 Da [5]. Individual LPS molecules on bacterial surface are bound together by various forces, including electrostatic and hydrophobic interactions and hydrogen bonds. Bivalent metal cations, such as Mg^{2+} and Ca^{2+}, stabilize the membrane through interactions with anionic groups of the inner and outer LPS core. Chelating agents such as EDTA are known to remove these ions from the bacterial surface and increase the membrane permeability [1].

Enterobacterial LPS composition substantially determines the surface properties of the cells, which follows from the coincidence of the electrokinetic potential (EKP) values of LPS preparations and initial EKP values of the cells from which these preparations were isolated. The study of EKP in *E. coli* and *Salmonella* cells of different chemotypes has shown that the maximal EKP values were observed in deep Re–Rd mutants. The minimal EKP values were observed in S chemotype cells carrying full O-polysaccharide chains, which shield the surface charge [6]. The nature of the changes of EKP values in R mutants, associated with the change of core composition, is little studied.

Obviously, the EKP value of the cells is determined not only by the composition of LPS molecules but also by the number of these molecules on the cell surface. Previously, it has been shown that pretreatment of *E. coli* K-12 cells by EDTA causes a decrease of the surface charge of the cells [7] due to partial release of LPS molecules from the cell wall.

The charged groups of outer membrane proteins may also contribute to the total surface charge of the cells. The surface charge formed by structural cell wall components influences the formation of colonies, adhesive properties of bacterial cells, their interaction with

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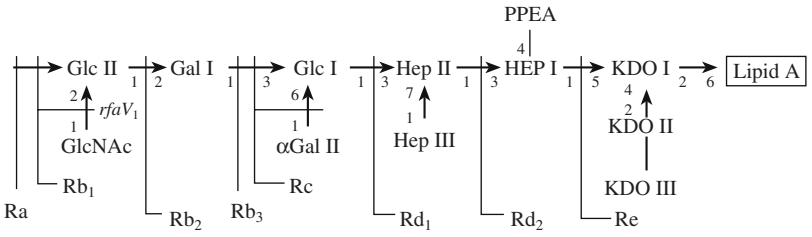

Fig. 1. The structures of LPS cores from *E. coli* K-12 (from Ra to Re) (from Reeves, 1994, adapted).

antibiotics, detergents, and biologically active molecules [8]. Previously, it has been shown that the cell surface LPS composition of different *E. coli* strains affects penetration of lysozyme and low-molecular antibiotics assessed by the change of optical density of cell suspensions [9].

The surface properties of the cells are most often studied by the method of cell electrophoresis that allows highly accurate characterization of the boundary between the cell and the environment [10].

The goal of the present work was to study the nature of changes of the surface electric properties of the cells, depending on the core structure of LPS molecules, by the method of cell microelectrophoresis and determination of cell sensitivity to lysozyme in two strains of *E. coli* K-12.

MATERIALS AND METHODS

Two strains of *E. coli* K-12, wild type D21 from the Russian Collection of Microorganisms (IBPM Russ. Acad. Sci.) and JM 103 from the German Collection of Microorganisms (DSMZ GmbH, Braunschweig, Germany), were used in the work. According to the classification of Boman and Monner based on the analysis of carbohydrate and fatty acid composition of the LPS of *E. coli* K-12 mutants, strain JM 103 is designated as D21f2 [11]. The cells of *E. coli* K-12 D21 and *E. coli* K-12 JM 103 were grown on an agarized M9 medium and 382 medium (DSMZ GmbH, Braunschweig, Germany), respectively, at 37°C for 24 h. The content of Ca^{2+} and Mg^{2+} ions in the media was adjusted to equal values, since these ions affect both cell wall formation in the course of bacterial growth and LPS release from the cell wall [12].

For electrokinetic measurements, the cells were washed off the agar surface and washed twice with phosphate–citrate buffer, ionic force 0.02, pH 7.0 [13], followed by preparation of cell suspensions with the required pH value at a concentration of 5×10^6 cells/ml. Electrophoretic mobility (EPM) of 25 cells was measured in a Parmoquant-2 microscope (Carl Zeiss, Jena, Germany) at 20°C. The EKP of the cells was calculated by the formula of Smoluchowskii [10]. The validity of results was checked by the Student–Fisher's criterion.

Before the treatment with lysozyme, the cells were washed off the surface of agarized medium by phosphate buffered saline (PBS), pH 7.4, and their concentration was adjusted to 10^8 cells/ml. After centrifugation and rinsing with PBS, the cells were resuspended in the same buffer containing lysozyme in concentrations of 150–1000 μg/ml. The control cells were resuspended in pure PBS. The cells with lysozyme were incubated at 37°C for 1 h under gentle stirring.

To assess the effect of hypotonic shock on the control and lysozyme-treated cells, the cells were precipitated by centrifugation and washed twice with double-distilled water (pH 6.0). The degree of cell lysis and aggregation after incubation with lysozyme was assayed as the change in absorbance of cell suspensions measured by a FEK-56M photocolorimeter (Russia) at 540 nm in a 1-cm cuvette.

LPS release at the interaction of bacteria with lysozyme was studied at a concentration corresponding to an acute phase of inflammation (150 μg/ml). After incubation of cells with lysozyme under the above conditions, followed by centrifugation, the LPS content was determined in the supernatant by spectroscopy using a carbocyanine dye [14]. The spectra were registered in a spectrophotometer (Hitachi, Japan). The absolute quantity of released LPS in the sample was determined by the curves of absorption dependence of LPS–carbocyanine complex on LPS concentration at 468 and 464 nm for Ra- and Re-LPS, respectively. LPS from *E. coli* was isolated by the method proposed by Galanos [15].

RESULTS AND DISCUSSION

The studied *E. coli* strains are of the R chemotype (their LPS lacks O-antigen) and differ in the LPS core structure. LPS from *E. coli* D21 has a complete Ra core, while LPS from *E. coli* JM 103 has a minimally structured Re core (Fig. 1). The core composition, the negative charges of phosphate groups of lipid A, carboxyl groups of KDO (3-deoxy-D-*manno*-octulosonic acid), and phosphate groups of phosphorylated sugars of the core affect the charge of the LPS molecule and the total negative charge of a cell. Membrane-bound proteins can make their own contribution to the surface charge [16]. The surface groups of cell wall components may be ionized and thus determine pH dependence of the cell surface charge.

Figure 2 shows the dependence of EKP of *E. coli* D21 and *E. coli* JM 103 cells on pH of the medium. The shapes of the curves are typical of bacterial cells with the acid–base type of surface [10]. The cells of both *E. coli* strains had similar EKP values in the pH range of 7.0–7.8. Reliable differences in the cell EKP values between the strains were observed only at more acidic pH values of the suspension (5.2–4.6). In this case, the cells of *E. coli* JM 103 had a higher negative charge of the cell surface than the cells of *E. coli* D21. At further pH decrease of the cell suspensions, the cells gained positive EKP values after transition to isoelectric state, which could be indicative of the presence of basic groups, e.g., amino groups of porins, on their surface. In acidic medium, the phosphate groups of lipid A and heptose residues as well as the carboxyl groups of KDO (2-keto-3-deoxyoctanoute) are completely protonated; therefore, only the basic groups of proteins remain charged at low pH values.

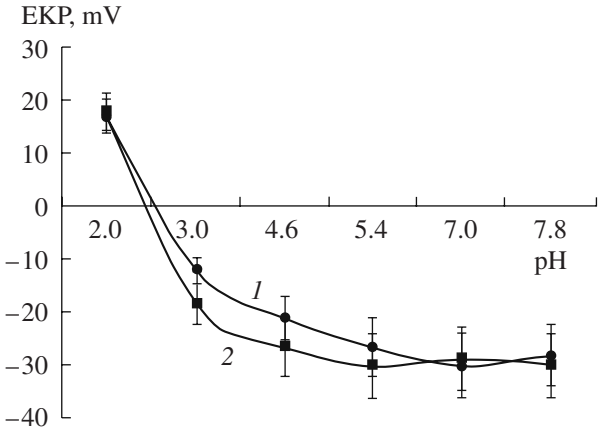

Fig. 2. Dependence of EKP of *E. coli* D21 (*1*) and *E. coli* JM 103 (*2*) cells on pH of the medium.

The measurement of cell EPM of the studied *E. coli* strains showed aggregation of cells at pH 2–3 due to the decrease of their negative surface charge and, consequently, electrostatic repulsion. Visual microscopic analysis revealed larger aggregates in the suspension of *E. coli* D21 cells. Aggregation complicated the process of measuring EPM of the cells. The presence in these suspensions of single cells with the surface close to isoelectric state considerably increased the spread of measured values. This fact made it difficult to reveal a reliable difference between the positive EKP values of cells of the studied strains. However, the higher pH of *E. coli* D21 cell surface transitions to the isoelectric state (Fig. 2) and higher aggregation as compared with the cells of *E. coli* JM 103 suggests that D21 cells have more proteins on their surface.

As is known from the literature, the outer membrane of deep R mutants contains much less protein per unit of the surface as compared with the S chemotype of bacteria [16]. Our results show that the R chemotype bacterial strains different in core structure also have different amounts of protein on their surface. The strain *E. coli* JM 103 (Re mutant) has less protein than the strain *E. coli* D21 (Ra mutant).

The core composition determines the size of LPS molecule and affects its charge value, which may be of critical importance for the interaction of cells with the environment. LPS from *E. coli* D21 comprises two additional phosphate groups of phosphorylated sugars in the inner core, in contrast to *E. coli* JM 103, which, along with the carboxyl groups of KDO and phosphate groups of lipid A, contribute to the total negative charge on cell surface [17]. Basing on the core composition of the LPS from *E. coli* D21 cells, it could be expected that their cell surface would be more electronegative. The electrokinetic measurements of cells of the two strains at pH 4.6–5.2, at which the differences in EKP are most pronounced, showed that the absolute EKP values of *E. coli* JM 103 cells were reliably higher than those of *E. coli* D21 cells (Fig. 2). These results suggest that the higher negative charge on the cell surface of strain *E. coli* JM 103 may be due to the higher, as compared with *E. coli* D21, quantity of LPS in the cell wall and, consequently, their more tight packing in the outer membrane.

It is known that a decrease in the total charge of an LPS molecule results in a decrease of repulsion and hydration of lipid A head groups, which assumes more tight packing of LPS molecules in the aggregate [18]. The Re-LPS molecule from *E. coli* containing only two or three phosphate groups is much more rigid than Ra-LPS containing about seven phosphate groups [19]. The neighboring molecules of lipid A can be exposed to a strong intermolecular interaction, because only a few phosphate groups are present. This interaction weakens in Re-LPS due to the additional negative charge of the KDO residues. Further increase of the negative charge, due to the presence of phosphate groups of heptose residues, requires the presence of bivalent cations for the charge balance and maintenance of stability. These arguments suggest that more truncated LPS composition promotes stronger interaction between the neighboring molecules and tighter packing in the membrane, especially in the absence of additional bivalent cations, as in our experiments.

Our supposition of the changing quantity and density of packing of the LPS molecules on the cell surface depending on the core length was tested in experiments with lysozyme treatment of the cells of the studied strains. After incubation of the bacteria with lysozyme, the cells were exposed to hypotonic shock, i.e., resuspended and washed in double-distilled water. It was revealed that the treatment of cells by lysozyme decreased the optical densities of suspensions as compared with the control cells incubated in PBS buffer. The revealed effect is probably associated with the partial cell lysis and aggregation after incubation with lysozyme. Figure 3 shows the change of optical densities of cell suspensions of the studied strains treated

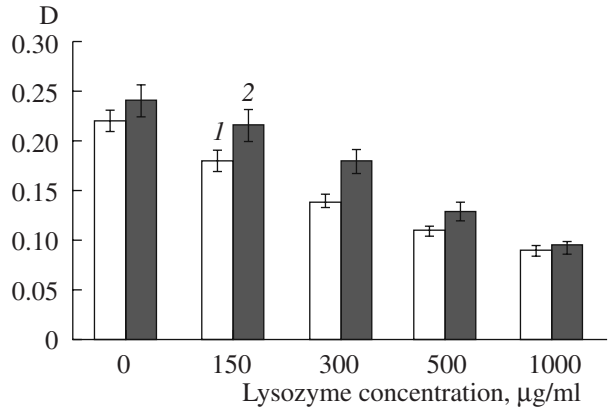

Fig. 3. The change of optical density of *E. coli* D21 (*1*) and *E. coli* JM 103 (*2*) cell suspensions treated with different concentrations of lysozyme and exposed to hypotonic shock.

with different concentrations of lysozyme and exposed to hypotonic shock. It can be seen that the process of optical density decrease at lysozyme concentrations of 150–300 μg/ml was faster in the cell suspension of strain D21. Hence it follows that cells with the complete LPS core are more sensitive to hypotonic shock after the action of lysozyme.

The effect of lysozyme on different *E. coli* strains was assayed by LPS release from bacterial cell walls. The table shows the results of LPS release from the cell wall after incubation of cells in PBS buffer and in the medium containing 150 μg/ml of lysozyme. As follows from the data presented in the table, the quantity of LPS released from the control cells incubated in PBS buffer depends on bacterial LPS composition and increases with the core extension. LPS release from the cell wall into the medium in the control cells is determined by cell wall instability under experimental conditions, when PBS buffer was used without the addition of Ca^{2+} and Mg^{2+} ions [11]. The quantity of LPS molecules released from bacteria incubated with lysozyme was higher in cells with the Ra core of LPS than in cells with the Re core. Less pronounced LPS release from the cells with Re type LPS as compared with the Ra core cells under the action of lysozyme can be explained also by a different density of LPS packing in the cell wall of bacteria with different LPS core compositions. For destruction of the peptidoglycan layer, lysozyme has to permeate through the outer leaflet of the outer membrane, which is less permeable in the cells with more dense LPS packing, which we expect in bacteria with Re type LPS. The results demonstrate that the truncated composition of LPS molecules provides their tighter packing in the cell membrane, which conforms to the data on EKP of the cells (Fig. 2). The presence of a complete oligosaccharide core (Ra type) makes LPS packing looser, providing easier LPS release from the cell wall. Since the mechanism of lysozyme penetration to the peptidoglycan layer across the outer bacterial membrane has been studied insufficiently, its penetration into the cell through cell wall protein channels should not be ruled out. In this case, the smaller effect of lysozyme on the cells with Re LPS is due to the smaller quantity of protein on their surface as compared with the bacteria containing Ra LPS in their cell wall.

The effect of PBS and lysozyme on LPS release from the cell wall of *E. coli* D21 and *E. coli* JM 103

Medium	Protein concentration, μg/ml	Quantity of LPS released from cells, μg/ml	
		Re chemotype *E. coli* JM 103	Ra chemotype *E. coli* D21
PBS buffer	–	3.50 ± 0.25	6.30 ± 0.75
PBS buffer + lysozyme	150	3.85 ± 0.30	8.82 ± 0.92

Thus, the results of electrokinetic measurements of *E. coli* K-12 cells of two R chemotype strains with well-characterized LPS core composition and studies of the interaction of these cells with lysozyme suggest that the higher negative value of the surface charge of *E. coli* JM 103 cells (Re type LPS) is determined by the higher quantity and density of packing of LPS in the cell wall as compared with the cells of *E. coli* D21 (Ra type LPS).

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