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The Electrokinetic Surface of Five Enteropathogenic *Escherichia coli* Serogroups

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ABSTRACT

The surface ionogenicity of five enteropathogenic *Escherichia coli* serogroups (O111:H2, O111:H12, O125:H9, O119:H6, and O26:H11) was investigated by electrokinetical approaches. All of the studied surfaces are negatively charged with their mean values of zeta potential (ZP) varying from −9.0 (O26:H11) to −11.9 mV (O111:H2). The populational behavior of the all bacteria are similar since very high ZP values varying from −26 to −30 mV were obtained in experiments carried out with the slip plane calculated at 6.83 nm from the cell surface. All the surfaces are extremely acidic, because the isoelectrophoretic points are localized at pH values below 3.0. Treatment of the microorganisms with neuraminidase did not alter their surface anionogenicity, while treatment with trypsin or phospholipase C reduced their negative charge.

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Index Entries: *Escherichia coli;* EPEC; cell surface; surface charge.

INTRODUCTION

It has been proposed that most of the enteropathogenic *Escherichia coli* (EPEC) serogroups interact with HeLa and Hep-2 cells by a process in which localized adherence (LA) is involved (*1–3*). This adherence pattern is mediated by an adhesion molecule class called EPEC adherence factor (EAF), and a DNA probe has been used to detect *E. coli* strains bearing the genes codifying EAF (*4*).

Ultrastructural studies of the intestinal mucosa from infected humans and animals suggest that EPEC induces an attaching-effacing lesion in such mucosa (*5–8*). In this lesion, many bacteria have been found destroying the brush border of enterocytes and intimately adhering to the apical epithelial surface causing cupping and pedestal formation in the host cell surface. In regions of bacterial attachment, the cytoskeletal components are destroyed or disrupted. In addition, it has also been pointed out to the role played by EAF molecules in the initial steps of the interaction process of *E. coli* and the host mucosa (*9*).

Bacterial adhesion is indeed a prerequisite for the initial colonization of the host tissue. The interaction between bacteria and their host cells is a phenomenon which obviously includes many specific and nonspecific effects. Among the nonspecific effects, surface properties of physico-chemical nature are of course involved. Two properties which will determine whether or not adhesion will take place are the nature and magnitude of the eletrostatic surface charge (*10–12*). Specific chemical groups may contribute to the net cell surface charge such as acidic groups from glycoproteins, sulphates from glycosaminoglycans, and phosphates from phospholipids (*13*).

The aim of this work is to determine and compare the surface charge of five EPEC serogroups.

MATERIAL AND METHODS

Bacterial Strains

E. coli strains isolated from children with diarrhea were serotyped and probed for genes that code for EAF. Briefly, the microorganisms

showing a localized adherence to both HeLa and Hep-2 cells were used throughout (*14–16*). In this study, five strains of these serotypes were selected according to their respective hybridization with the EAF probe and percentage of isolation in a previous epidemiological survey (*17*): O111:H2, EAF+ (17.6%); O125:H9, EAF− (16.8%); O111:H12, EAF− (11.8%); O119:H6, EAF+ (7.6%); and O26:H11, EAF− (6.7%).

Cell Electrophoresis

Two alternative methods were used: measurements of individual bacteria through electrophoretic mobility (EPM) determinations and populational analysis by means of direct measurements of their zeta potential (ZP). The EPM was determined in a Zeiss cytopherometer by timing the passage of cells through a calibrated graticule when a current of 6 mA and a gradient of 4.9 V/cm was applied to the electrophoresis chamber. Cells were timed in alternative directions to minimize electrode polarization. Measurements were made on at least 50 individual cells which were suspended in NaCl solution (ionic strength 0.145 mol/dm^3, pH 7.2 at 25°C). The EPM was calculated by using the following equation: $d/t \times D/V$, where d is the distance (in μm), covered by the cells during measurements (usually 16 μm), t is the time (in seconds) required by the cell to cover the distance d, D is the distance between the two electrodes (18 cm) and V is the potential applied to the electrodes. Values of EPM were converted to ZP by using the formula ZP = 12.85 EPM (in millivolts) (*12*). The measurements of ZP of the microorganisms were carried out by using a PenKen Laser-Zee System (Model 501). Typical performance parameters for the grating analyzer system were as follows: chamber volume of approx 15 mL, voltage of 100 V, measurement time of about 2–3 s, temperature of the cell suspension of 25°C, with the movement of the bacteria monitored with a binocular microscope during the measurements. Values of ZP are expressed in millivolts. For all determinations, bacterial suspensions were harvested in logarithmic growth phase. In some experiments the ionic strength of the solution in which the cells were suspended was varied from 0.00218–0.145*M*. This variation in ionic strength (I) corresponds to a variation in the thickness of the ionic atmosphere (debye-length-1/K, given in nanometers) that is calculated according to the equation: $1/K = 3.05/I^{1/2}$ Å. In other experiments, the pH of the solution was varied from 3.0–11.0, with the ionic strength maintained at 0.145 mol/dm^3.

Enzymatic Treatments

Neuraminidase

Cells were collected by centrifugation, washed twice in phosphate-buffered saline (PBS), resuspended in PBS (1×10^8 cells/mL), and then incubated for 60 min at 37°C with 0.1 U/mL neuraminidase from *Clostridium perfringens* (Type X, Sigma Chemical, St. Louis, MO) previously dissolved in Tyrode's solution, pH 6.0. Incubation was interrupted by the addition of bovine serum.

Trypsin

Microorganisms were incubated for 60 min at 37°C in the presence of 1 mg/mL trypsin (Type III-S, Sigma) in PBS, pH 7.2. Incubation was interrupted by addition of fetal bovine serum.

Phospholipase C

Cells were collected, washed, incubated for 60 min at 37°C in the presence of 1 U/mL phospholipase C from *Clostridium perfringens* (Sigma), and diluted in Tris-Maleate buffer, pH 7.5. Incubation was interrupted by washing the cells with PBS, pH 7.2.

After each enzymatic treatment, the cells were collected by centrifugation, washed twice in PBS, and fixed in glutaraldehyde for determination of their ZP.

RESULTS

All of the given EPM were based on 50 individual measurements made on each experimental group. The EPM of the cells when measured at pH 7.2 and ionic strength of 0.145 mol/dm^3 varied with the age of the bacterial cultures (Fig. 1). Strains of *E. coli* collected from cultures in the stationary phase of growth had a net negative surface charge whose mean EPM were: −1.09 (O111:H12), −1.37 (O119:H6), −1.33 (O26:H11), −1.17 (O125:H9), −1.44 μm/s/V/cm (O111:H2) compared with −1.29 (O111:H12), −1.20 (O119:H6), −1.17 (O26:H11), −1.35 (O125:H9), and −1.35 μm/s/V/cm (O111:H2), for populations in the exponential phase of growth. Some strains had a low mean negative charge during the exponential phase, while others had the lowest during the stationary phase. Either glutaraldehyde fixed as unfixed bacteria had similar electrokinetical behavior with very close mean values ($p < 0.01$).

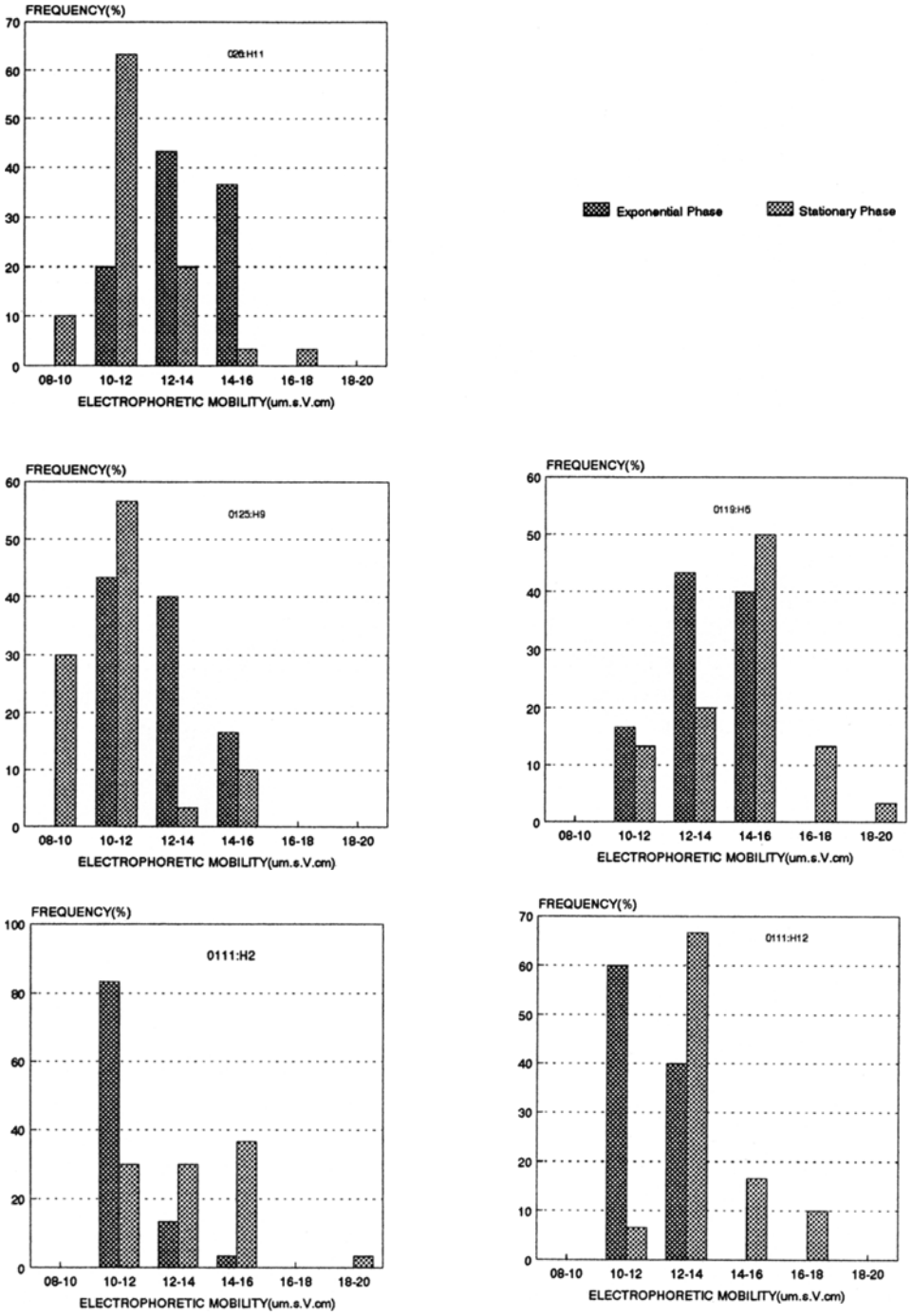

Fig. 1. Populational analyses of the electrophoretic mobility of *E. coli* serotypes obtained from stationary and exponential phases of growth.

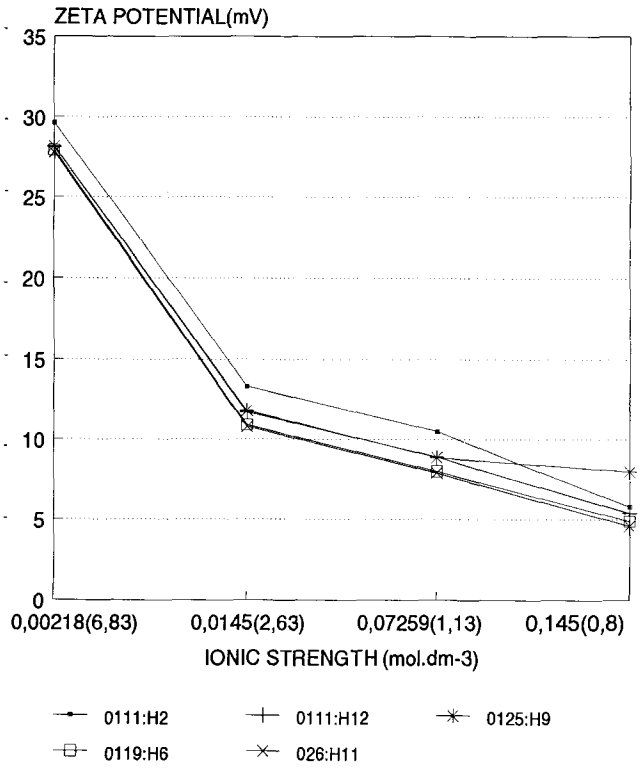

Fig. 2. Effect of the ionic strength on the ZP of *E. coli* serotypes.

Although all strains showed mixed populations, the heterogenicity in terms of surface charge was more evident in those microorganisms collected at the stationary phase of growth (Fig. 1). Thus, microorganisms from exponential phase of growth were used throughout.

The variation in the ionic strength of the solution where bacteria were suspended, had a marked influence on the ZP of the all *E. coli* strains (Fig. 2). As the concentration increases, the ZP decreases. At the lowest ionic strength, the net surface charge of the cells was more negative. However, as can also be seen in Fig. 2, all the microorganisms exhibited very close mean ZP values when the slip plane was placed at 0.8–2.63 and 6.53 nm, respectively.

The ZP curves obtained in experiments concerning pH variation resulted almost in a same electrokinetical behavior. It is important to point out that all these measurements were made in solutions whose ionic strength was maintained at 0.145 mol/dm^3. All the *E. coli* strains seem to possess predominantly carboxylic surfaces because at very low pH values they were fully ionized. The isoelectrophoretic points seem to be localized at pH below 3 (Fig. 3).

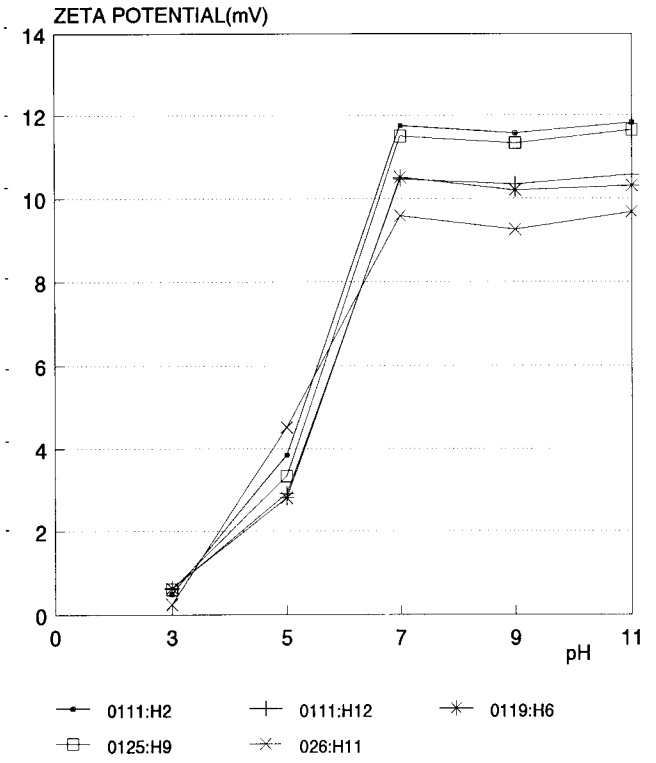

Fig. 3. Effect of the pH of the solution on the ZP of *E. coli* serotypes.

Table 1
Effect of Phospholipase C on the ZP of *E. coli* Serotypes

E. coli serotypes	ZP (in mV)		Variation (%) in ZP[a]
	Control	Enzyme treatment	
O111:H2	−11.9 ± 0.05	−10.02 ± 0.03	−25.2
O111:H12	−10.1 ± 0.07	− 8.9 ± 0.11	−14.8
O125:H9	−11.4 ± 0.04	− 8.8 ±0.08	−20.2
O119:H6	− 9.9 ± 0.04	− 9.1 ± 0.07	−11.1
O25:H11	− 9.8 ± 0.06	− 8.7 ± 0.04	−11.2

[a] Values were obtained from (ZPc-ZPe/ZPc) × 100, where the indexes "c" and "e" refer to control- and enzyme-treated microorganisms. Values higher than 10% are significant.

Treatment of the cells with trypsin and phospholipase C induced significant decreases in the ZP of the *E. coli* strains. Decreases were more effective with phospholipase C treatment (11.1–25.2%) (Table 1) than with trypsin (8.03–15.4%) (Table 2). In contrast, neuraminidase did not change the anionogenicity of the all investigated surfaces (0.19–1.4%) (Table 3).

Table 2
Effect of Trypsin on ZP of *E. coli* Serotypes

E. coli serotypes	ZP (in mV)		Variation (%) in ZP[a]
	Control	Enzyme treatment	
O111:H2	−11.9 ± 0.07	−10.1 ± 0.03	−15.4
O111:H12	−10.2 ± 0.03	− 8.74 ± 0.06	−14.3
O125:H9	−11.2 ± 0.04	−10.3 ± 0.09	− 8.03
O119:H6	− 9.6 ± 0.06	− 8.5 ± 0.08	−11.4
O25:H11	− 9.0 ± 0.02	− 7.82 ± 0.05	−13.1

[a] See Table 1 footnote.

Table 3
Effect of Neuraminidase on the ZP of *E. coli* Serotypes

E. coli serotypes	ZP (in mV)		Variation (%) in ZP[a]
	Control	Enzyme treatment	
O111:H2	−11.7 ± 0.07	−11.6 ± 0.04	−0.5
O111:H12	−10.4 ± 0.04	−10.38 ± 0.03	−0.2
O125:H9	−11.5 ± 0.05	−11.3 ± 0.03	−1.4
O119:H6	− 9.6 ± 0.04	− 9.64 ± 0.05	−0.4
O25:H11	− 9.6 ± 0.05	− 9.43 ± 0.06	−0.7

[a] See Table 1 footnote.

DISCUSSION

Our present observations show that the five EPEC serogroups have a negative surface charge as previously shown for most of the investigated prokaryotes (*11,12*). Measurements of the ZP of the microorganisms collected from different phases of growth clearly show that those obtained from cultures at exponential phase of growth are much more homogeneous in terms of surface charge than those found at stationary phase of growth. This behavior has also been found in some other bacteria (*18*) probably reflecting a high level of asynchronism among microorganisms from different phases of growth.

It has been shown that the net negative surface charge of most of the investigated eucaryotes is mainly a result of the dissociation of ionogenic surface groups as carboxyls from sialic acid residues associated to glycoproteins and glycolipids (*13,19,20*). Previous

studies have reported that sialic acids are the main surface anionic groups in erythrocytes (*21,22*), protozoa (*23*), fungi (*24*), and mamalian cells (*22*). In some species, sialated molecules have been associated with capsular antigens and LPS from outer membranes (*25,26*).

In the present study, neuraminidase treatment did not change the surface charge of the *E. coli* strains. However, we cannot exclude the possibility that sialic acid residues were present, but were not sensitive or accessible to the enzyme. Although trypsin treatment induced loss of negatively surface charge groupments more than phospholipase C, it was not as effective. These results suggest that phospholipase C and trypsin sensitive sites contribute to the negative surface charge of the *E. coli* strains and that differences exist between the surfaces of these bacteria from the different strains. We still are not able to infer if such surface differences are mainly related to quantitative or qualitative aspects.

Another result is the degree of anionogenicity among the five investigated *E. coli* strains. The clinical significance and relevance of such data deserves to be further investigated.

The data obtained from experiments performed with solutions at various pH regard cells as having carboxyl surfaces whose isoelectrophoretic (IEP) points are close to 2.6 (*12*). Unfortunately, we were not able to determine the IEP of the five analyzed *E. coli* strains. However, we suggest that these surfaces possess considerable ionization of strong acidic carboxyls because all the IEP seem to be localized below pH 3.0. In addition, the data shown in Figs. 2 and 3 indicate that the five *E. coli* strains are similar in qualitative terms since their electrokinetical behavior are closely related. Thus, it seems that the differences found in the mean ZP values may be related to the amount of ionogenic species among the *E. coli* strains in this study. In conclusion, our results indicate the possibility of a direct correlation between surface charge and virulence in enteropathogenic *E. coli* strains.

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