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Difference in surface properties between *Escherichia coli* and *Staphylococcus aureus* as revealed by electrophoretic mobility measurements

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Abstract

Electrophoretic mobilities of *Escherichia coli* and *Staphylococcus aureus* were measured in media of different pH values and ionic strengths at 310 K and the results were analyzed via a new mobility formula which was derived on the assumptions of uniform charge distribution in the cell surface layer of finite thickness and ion-penetrability in the layer. *E. coli* was shown to have a more negatively charged and less soft surface than that of *S. aureus*. It is suggested that electrophoretic mobility measurement can be used to detect the difference in surface structure between gram-positive and gram-negative bacteria.

Keywords: Electrophoretic mobility; *E. coli*; *S. aureus*; Surface charge density; Surface softness

1. Introduction

A knowledge of bacterial surfaces is very important because such events as flocculation and adhesion to solid surfaces of the cells are strongly dependent on their surface properties, especially on their surface hydrophobicity and surface electric properties. For this reason, many invesigations have been performed on the surface properties of a large variety of bacteria by means of contact angle measurement, electrophoresis, and X-ray photoelectron spectroscopy, etc. [1–7]. Nevertheless, our understanding of the surface properties of cells is still limited due to the complexity of cell surfaces and the lack of models which can be used to gain satisfactory information about the cell surface structure.

The outer cell membrane of gram-negative bacteria including *Escherichia coli* (*E. coli*) is known to be covered with a lipopolysaccharide layer of 1–3 μm thickness while the surface of gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) has a peptidoglycan layer on which teichoic acid, teichuronic acid, and proteins are covalently bound. In view of this, a considerably large difference in the electrophoretic behavior, and hence, if a proper mobility formula is employed to analyze the mobility data obtained, a significant difference in the surface properties is expected to be recognized between the two types of bacteria [8–11].

Although electrophoresis is a very convenient and useful technique to investigate the surface electrical

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properties of microparticles it should be noted that unless a proper mobility formula is adopted to analyze the experimentally obtained data we cannot draw satisfactory conclusions concerning the electric properties of particle surfaces. The Smoluchowski mobility formula is obviously unsuitable for our purpose since it was derived on the basis of a surface model in which the electric charges are located only at the ion-impenetrable particle surface of zero thickness. At the surface of biological cells the surface charges are distributed through an ion-penetrable layer of finite thickness. Hence, a new mobility formula is needed to analyze mobility data for biological cells.

Recently, Ohshima and Kondo derived an approximate mobility formula for microparticles with a surface charge layer which depends on a weighed average of the Donnan potential and the potential at the boundary between the surface charge layer and the surrounding medium [12]. This formula assumes a uniform charge distribution in an ion-penetrable surface layer of finite thickness and contains two undetermined parameters, charge density and resistance to liquid flow in the surface layer. Since the Donnan potential and the surface potential in the formula are ionic strength-dependent functions of the charge density in the surface layer, curve fitting is necessary to estimate the charge density from the mobility data as a function of the ionic strength of the medium at a fixed pH value using various values of charge density and resistance to liquid flow. The mobility formula has proved useful and successful in obtaining valuable information on the surface electric properties of biological cells [13–16] and synthetic microparticles with a structured surface [17–19].

This paper describes the results of an analysis of electrophoretic mobility data for *E. coli* and *S. aureus* in the media of various pH values and ionic strengths with the new mobility formula.

2. Experimental

2.1. Bacteria

E. coli and *S. aureus* were supplied by the Central Research Laboratory, Santen Pharmaceutical Co., Ltd., Osaka, Japan and kept on slopes of soybean casein digest agar (Difco, USA) at 277 K.

The organism was transferred into a small amount of the soybean casein digest agar (SCD) for preincubation at 310 K in a shaking water bath for 24 h. After preincubation, the culture was transferred into a large amount of SCD for incubation at 310 K in the water bath for 4 h. The culture was then centrifuged at 2220 *g* for 5 min so as to harvest the organism at the stationary phase and the sediment was collected, washed three times with buffer solution, and redispersed in the same buffer solution. The buffers used were acetate (pH 3.0, 4.0, 5.0, and 6.0), phosphate (pH 7.0 and 8.0), and carbonate (pH 9.0 and 10.0) solutions. The ionic strength of these solutions was varied from 0.005 to 0.154 M with addition of NaCl.

2.2. Measurements of electrophoretic mobility

The electrophoretic mobility of the organism in each of the buffer solutions was measured with a Pen Kem System 3000 automated electrophoresis apparatus (Pen Kem, Inc., New York, USA) at 310 K. The measurement was repeated at least 64 times (32 times in both directions) for each sample and the readings were averaged.

3. Results and discussion

3.1. Electrophoretic mobility

Figs. 1 and 2 show the electrophoretic mobility for *E. coli* and *S. aureus* as a function of the pH of the medium at different ionic strengths, respectively. Comparison of the two figures reveals the following facts: *S. aureus* is charged less negatively than *E. coli* at all pH values of the medium studied in this work, even though both organisms have a net negative charge in this pH region. It is also seen that the electrophoretic mobility of the gram-positive bacteria is less sensitive to changes in pH value and ionic strength than that of the gram-negative bacteria, being clearly indicative of a significant difference in surface structure between the two organisms. In addition, the mobility for both organisms never shows a tendency to converge to the zero value at high ionic

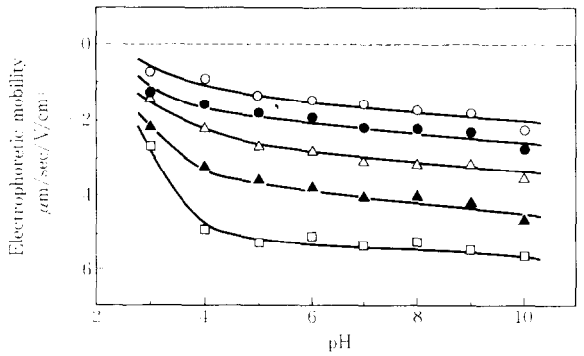

Fig. 1. Electrophoretic mobility of *E. coli* as a function of pH at ionic strengths of 0.154 (open circles), 0.10 (closed circles), 0.050 (open triangles), 0.025 (closed triangles) and 0.005 (open squares) (310 K).

strengths of the medium. This demonstrates the inapplicability of the Smoluchowski formula to analyze the mobility data for the organisms because the formula predicts the mobility to approach the zero value at high ionic strengths of the medium.

3.2. Analysis of mobility data

Analysis of mobility data was carried out using the following mobility formula [12]:

$$\mu = \frac{\epsilon_r \epsilon_0}{\eta} \frac{\psi_0/\kappa_m + \psi_{DON}/\lambda}{1/\lambda + 1/\kappa_m} + \frac{zeN}{\eta\lambda^2} \qquad (1)$$

where μ is the electrophoretic mobility, ϵ_r and ϵ_0 are the relative permittivity of the medium and the permittivity of a vacuum, respectively, η is the viscosity of the medium, ψ_0 is the potential at the boundary between the medium and the surface region, ψ_{DON} is the Donnan potential, κ_m is the Debye–Hückel parameter of the surface region, λ is a parameter characterizing the resistance to liquid flow in the surface region, z and N (m^{-3}) are the valence and the number density of charged groups expressed as the algebraic sum of positive and negative charges in the surface region, and e is the elementary charge. This formula holds only when the reciprocal of the Debye–Hückel parameter, $1/\kappa_m$, is negligibly small when compared with the thickness of the surface charge layer.

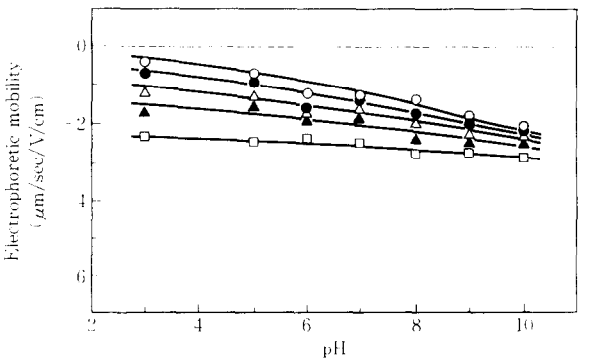

Fig. 2. Electrophoretic mobility of *S. aureus* as a function of pH at ionic strengths of 0.154 (open circles), 0.10 (closed circles), 0.050 (open triangles), 0.025 (closed triangles) and 0.005 (open squares) (310 K).

The surface potential, ψ_0, the Donnan potential, ψ_{DON}, the Debye–Hückel parameter in the surface region, κ_m, and the parameter characterizing the resistance to liquid flow in the surface region, λ, are given as follows:

$$\psi 0 = \frac{kT}{ve}\left(\ln\left\{\frac{zN}{2vn} + \left[\left(\frac{zN}{2vn}\right)^2 + 1\right]^{1/2}\right\} + \frac{2vn}{zN}\left\{1 - \left[\left(\frac{zN}{2vn}\right)^2 + 1\right]^{1/2}\right\}\right)$$

$$\psi_{DON} = \frac{kT}{ve}\ln\left\{\frac{zN}{2vn} + \left[\left(\frac{zN}{2vn}\right)^2 + 1\right]^{1/2}\right\} \qquad (2)$$

$$\kappa_m = \kappa\left\{\left(\frac{zN}{2vn}\right)^2 + 1\right\}^{1/4}$$

$$\kappa = \left(\frac{2nv^2e^2}{\epsilon_r\epsilon_0}\right)^{1/2}, \quad \lambda = \left(\frac{\gamma}{\eta}\right)^{1/2}$$

where v and n (m^{-3}) are the valence and the number concentration of the salt in the medium, κ is the Debye–Hückel parameter of the medium, and γ is the frictional coefficient of the surface charge layer.

Eq. 1 was used to perform curve fitting for the mobility data obtained at different pH values and ionic strengths. Thus, the best-fit values of N and λ were found at each of the given pH values and the highest ionic strength and then the mobility curve was drawn as a function of medium ionic strength at

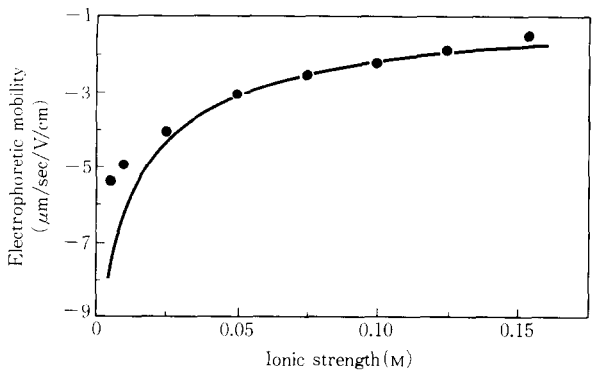

Fig. 3. Electrophoretic mobility–ionic strength curve for *E. coli* at pH 7.0. Closed circles indicate the experimentally observed mobility values and the solid line is the best fitted curve.

each pH. The best-fitted curves at pH 7.0 for *E. coli* and *S. aureus* are shown in Figs. 3 and 4, respectively. In both cases, the agreement between the experimentally determined and calculated mobility values is quite good at ionic strengths above 0.05 M, below which the equation gives somewhat more negative mobility values than the observed mobility values. A similar tendency was found at pH values other than 7.0. The values of N and λ that give the best fit are shown in Table 1 for *E. coli* and in Table 2 for *S. aureus*, respectively. The value of N increased and that of λ decreased with increasing medium pH for both *E. coli* and *S. aureus*, probably reflecting increases in the degree of dissociation of the acidic groups and liquid penetration in the surface region at high pH values.

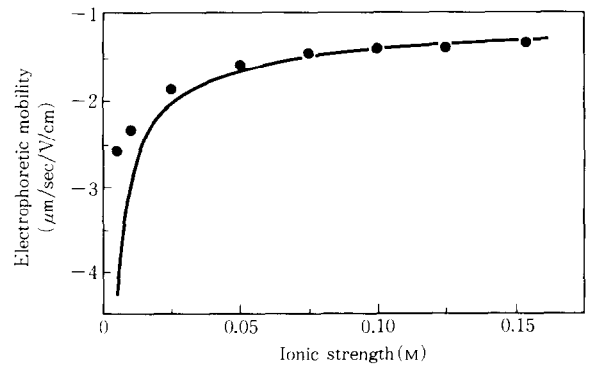

Fig. 4. Electrophoretic mobility–ionic strength curve for *S. aureus* at pH 7.0. Closed circles indicate the experimentally observed mobility values and the solid line is the best fitted curve.

Table 1
Values of N and λ that give the best fit for *E. coli*

pH	N (M)	λ (1/nm)
3.0	0.075	2.0
5.0	0.135	1.8
7.0	0.145	1.5
9.0	0.150	1.4

The fact that the new mobility formula represents well the observed mobility values at ionic strengths above 0.05 M indicates a uniform charge distribution and a constant resistance to liquid flow in the outer part (less than 1.5 nm thickness) of the surface charge layer for both organisms at all pH values used in the present work, because the formula has been derived on the assumptions of constant N and λ at a given pH. Hence, as far as this part of the surface charge layer is concerned, we can safely conclude that *E. coli* have a more negatively charged and more rigid surface than *S. aureus* based on the values of N and λ in Tables 1 and 2. Here, we wish to emphasize the significance of using both N and λ to characterize the surface properties of biological cells. So far, only N has been successfully used to gain information on the charge distribution in the surface region of red cells [13,16], lymphocytes [14], and white cells [15]. However, recent work on the electrophoretic behavior of certain lymphosarcoma cells and their variant cells has strongly suggested the usefulness of λ as a measure of the surface softness of biological cells [20]. If λ is large the cell surface is hard while the cells have a soft surface when λ is small. This way, we can characterize the surface of biological cells in terms of N and λ.

There are several possible reasons why the observed mobility values deviate from the theoretical mobility curve at ionic strengths lower than 0.05 M. Firstly, as in the case of blood cells [13–16], it is possible that the charge density is not uniform deep inside the surface layer. In fact, the positive charges

Table 2
Values of N and λ that give the best fit for *S. aureus*

pH	N (M)	λ (1/nm)
5.0	0.023	0.67
7.0	0.025	0.53
9.0	0.030	0.50

arising from the basic groups of the proteins and peptides in the surface layer of the organisms [8–11] would reduce the charge density by neutralizing a part of the negative charges on the acidic groups of lipopolysaccharides and teichoic and teichuronic acids at low ionic strengths, where the charges located deep in the layer can contribute to mobility. Secondly, the resistance to liquid flow may change with the distance from the boundary between the surface layer and the surrounding medium since the bacterial wall is known to have a multilayered structure [8–11]. The third and most likely possibility is that both charge density and resistance to flow change deep inside the surface layer, in view of the multilayered surface structure of the organisms. Hence, the model used in the present work is not sufficient and a more complicated multisublayer model is needed to fully understand the electrophoretic behavior of bacteria.

In conclusion, the present study suggests that electrophoresis can be used to detect the difference in surface structure between gram-positive and gram-negative bacteria if the experimentally obtained mobility data are analyzed with a proper mobility formula.

Acknowledgements

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