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Low-frequency dielectric dispersion of bacterial cell suspensions

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ABSTRACT

Dielectric spectra of *Escherichia coli* cells suspended in 0.1–10 mM NaCl were measured over a frequency range of 10 Hz to 10 MHz. Low-frequency dielectric dispersion, so-called the α-dispersion, was found below 10 kHz in addition to the β-dispersion, due to interfacial polarization, appearing above 100 kHz. When the cells were killed by heating at 60 °C for 30 min, the β-dispersion disappeared completely, whereas the α-dispersion was little influenced. This suggests that the plasma (or inner) membranes of the dead cells are no longer the permeability barrier to small ions, and that the α-dispersion is not related to the membrane potential due to selective membrane permeability of ions. The intensity of the α-dispersion depended on both of the pH and ionic strength of the external medium, supporting the model that the α-dispersion results from the deformation of the ion clouds formed outside and inside the cell wall containing charged residues.

1. Introduction

Bacterial cells have been studied by dielectric spectroscopy to understand the electrical and structural properties of the plasma membrane, cell wall and cytoplasm. Fricke et al. [1] first reported dielectric dispersion of *Escherichia coli* cell suspensions in the frequency range of 100 kHz to 10 MHz, and suggested the presence of the insulating plasma membrane, of which specific capacitance was estimated to be 0.7 μF/cm^2. The dielectric dispersion, which is referred to as the β-dispersion, is due to interfacial polarization [2–4]. Carstensen and co-workers [5–8] subsequently revealed that bacterial cells have a conducting cell wall outside the insulating plasma membrane, and modeled the cells as a sphere covered with two concentric shells that correspond to the plasma membrane and the cell wall. The conductivity of the cell wall in low salt media is dominated by the mobile counterions of fixed charges in the cell wall. The spherical cell model was extended to ellipsoidal cell models, which were applied to the β-dispersion of rod-shaped *E. coli* cells [9,10].

Dielectric dispersion, termed the α-dispersion, was found for *Micrococcus Lysodeikticus* cells at frequencies below 10 kHz [11,12]. The α-dispersion, however, has not been reported except a few studies [13–15] because electrode polarization (EP) effects interfere with its accurate measurement. The origin of the α-dispersion is therefore still a controversial issue although the α-dispersion has been believed to be caused by the deformation of the counterion cloud outside the charged cell surface, i.e., counterion polarization (CP), from analogy with the low-frequency (LF) dispersion of charged colloids. Schwarz [16] proposed a condensed counterion model to interpret the LF dispersion of charged insulating particles suspended in electrolyte solutions. In the model, counterions move along the particle surface without exchanging with ions in the bulk phase. Einolf and Carstensen [12] extended the Schwarz model to analyze the α-dispersion of bacterial cells by taking into account both counterion layers inside and outside the cell wall. Afterward, Dukhin and Shilove [17] proposed a different model that includes diffusion of ions in the bulk phase and exchange of ions between the electrical double layer and the bulk phase. Many theoretical advances have been made along the Dukhin–Shilove model, as described in a review of Grosse and Delgado [18]. Grosse and Zimmerman [19] have extended the Dukhin–Shilove model to lipid vesicles and biological cells, which are modeled as a sphere covered with a single shell possessing charged surfaces, and have obtained an analytical solution that enables us to simulate the whole dielectric spectrum including the α- and the β-dispersions. Further, numerical simulation has been carried out with the cell model that has a porous charged wall outside the plasma membrane [20].

Recently, similar but alternative models have been proposed to interpret both of the α- and the β-dispersions of cell suspensions [21–26]. The models focus on the membrane potential evoked in living cells. Charges are accumulated at the membrane interfaces according to the potential difference across the membrane and move on the cell surface when an electric field is applied to

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the cell. The charge migration gives rise large polarization at low frequencies, resulting in the α-dispersion. The theoretical models imply that the membrane potential can be determined from the α-dispersion. Indeed, attempts to determine the membrane potential of *E. coli* cells were made [14,15]. Experimental evidence, however, is insufficient to support the relationships between the membrane potential and the α-dispersion.

In this article, the origin of the α-dispersion in *E. coli* cell suspensions has been studied. Dielectric measurement has been carried out not only to determine whether the α-dispersion is associated with the membrane potential in living cells but also to evaluate both contributions of the density of charged groups in the cell wall and the ionic strength of the external medium.

2. Materials and methods

2.1. Cultivation and preparation of E. coli cells

A strain (K12) of *E. coli* was obtained from the Institute for Fermentation, Osaka. Cells were grown in a culture medium containing 10% polypeptone, 2% yeast extract and 1% $MgSO_4{\cdot}7H_2O$ at 30 °C under aerobic conditions and were collected in the early stationary phase. The cells were washed at least twice in 1 mM NaCl.

Heat-treated cells were obtained by incubating intact cells in 1 mM NaCl at 60 °C for 30 min. After the heat treatment, the cells were washed in 1 mM NaCl and were used for examining the effects of the pH and NaCl concentration of the external medium. The pH of the cell suspensions was varied between 4 and 10 by titrating with 0.1 M HCl and 0.1 M NaOH. The NaCl concentration of the medium was changed from 0.1 to 4 mM.

2.2. Dielectric spectroscopy

Dielectric measurements have been carried out with a 4192A Impedance Analyzer over a frequency range of 10 Hz to 10 MHz. A conventional parallel plate capacitor similar to that described previously [27] was used as a measurement cell having a cylindrical sample cavity (4 mm in diameter and 4 mm in length) with platinum-black electrodes that were carefully prepared to reduce the EP effect. The measurement cell was filled with a cell suspension and its admittance was measured at 24–26 °C. Using the stray capacitance and the cell constant of the measurement cell, the admittance was converted into the relative complex permittivity ε^* of the suspension defined as $\varepsilon^* = \varepsilon + \kappa/(j2\pi f\varepsilon_0)$, where ε is the relative permittivity, κ is the conductivity, f is the frequency of the applied ac field, ε_0 is the permittivity of vacuum and j is the imaginary unit.

3. Results and discussion

3.1. Dielectric spectra of E. coli cell suspensions

Dielectric spectra were measured for cells suspended in 1–10 mM NaCl solutions at a volume fraction of 0.1–0.2. The osmolarity of the medium was not adjusted because the dielectric spectra were not influenced by addition of 0.3 M mannitol. Fig. 1 shows the relative permittivity of the cell suspensions measured at the same cell concentration. The low-frequency (LF) and the high-frequency (HF) dispersions, which correspond to the α- and the β-dispersions, respectively, are clearly seen although the EP effect covers the LF dispersion partly for 1 mM NaCl and mostly for 10 mM NaCl. The dielectric spectra including the EP effect were well represented by

$$\varepsilon^* = \varepsilon_h + \frac{\Delta\varepsilon_L}{1+(jf/f_L)^{\beta_L}} + \frac{\Delta\varepsilon_H}{1+(jf/f_H)^{\beta_H}} + \frac{\kappa_l}{j2\pi f\varepsilon_0} + Af^{-m}, \quad (1)$$

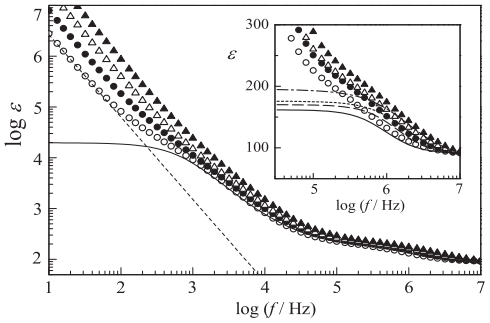

Fig. 1. Frequency f dependence of the relative permittivity ε of *E. coli* cells suspended in 1 (○), 3 (●), 6 (Δ) and 10 (▲) mM NaCl. The cell suspensions were prepared to have the same cell concentration. The best-fit curve (solid line) for the data with 1 mM NaCl is shown separately from the EP effect (broken line). Inset: an expanded view of the HF dispersion with the best-fit curves for the data with 1 mM (solid line), 3 mM (broken line), 6 mM (dotted line) and 10 mM (dashed-dotted line).

which contains two Cole–Cole relaxation terms (subscripts L and H refer to the LF and the HF relaxation processes) and the EP term with constants A and m [28]. $\Delta\varepsilon_x$, f_x and β_x (x = L and H) are the intensity, characteristic frequency and broadening factor of the Cole–Cole relaxation, respectively. ε_h and κ_l are the HF limit of the relative permittivity and the LF limit of the conductivity, respectively. The values of ε_h, $\Delta\varepsilon_L$, f_L, β_L, $\Delta\varepsilon_H$, f_H, β_H, A and m in Eq. (1) were determined by the non-linear least squares method to minimize the residual χ^2

$$\chi^2 = \sum_i \{\log_{10} Re[\varepsilon^*_{ob}(f_i)] - \log_{10} Re[\varepsilon^*_{th}(f_i)]\}^2, \quad (2)$$

where $Re[\varepsilon^*_{ob}(f_i)]$ and $Re[\varepsilon^*_{th}(f_i)]$ are the real parts of the observed and theoretical complex permittivities, respectively, and f_i is the ith frequency. The best-fit parameters are listed in Table 1. The values of κ_l and the medium conductivity κ_a were estimated from the conductivities of the cell suspension and the external medium at 0.1–1 kHz, respectively.

The $\Delta\varepsilon_H$ of the HF dispersion increased with increasing the NaCl concentration c_a of the medium (see the inset of Fig. 1 and Table 1). The ratio κ_l/κ_a increased as decreasing c_a, suggesting that the effective conductivity κ_p of the cell remains a high level while κ_a decreases. The high κ_p results from the high cell wall conductivity that is due to the mobile counterions of the fixed charges in the cell wall [8]. The presence of the conducting cell wall reduces the charging of the plasma membrane, resulting in the decrease in $\Delta\varepsilon_H$ [4,9]. The f_H increased with κ_a, which is interpreted in terms of interfacial polarization [9].

The values of $\Delta\varepsilon_L$ and f_L of the LF dispersion were unchanged between 1 mM and 3 mM. Above 6 mM, those include large errors in estimation owing to the EP effect, which makes quantitative discussion difficult. The value of β_L was about 0.85 and was comparable to that obtained for the α-dispersion of *M. lysodeikticus* cells ($\beta_L \approx 0.8$) [11]. The value of β_L was smaller than that of β_H, indicating that the LF dispersion distributed over a wider frequency range than the HF dispersion.

3.2. Effects of heat treatment

Several authors [21–26] proposed models in which the potential difference across the membrane plays an important role in the LF dispersion. The membrane potential is mainly resulted from the diffusion of specific ions along their concentration gradient produced by active transport, diminishing in dead cells. To examine whether the membrane potential takes part in the LF dispersion, the dielectric spectra of live cells were compared, at the same cell concentration, with those of cells killed by heating at 60 °C for 30 min