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Electric polarizability changes during *E. coli* culture growth

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ABSTRACT

The electric polarizability of bacteria has two main components: surface-charge dependent (SChD) and Maxwell–Wagner (MW). It has been reported that the low frequency SChD component of *Escherichia coli* K12 still arise in the frequency range 20 kHz – 2 MHz, together with the high-frequency MW one. All the previous experiments were carried out with bacterial cultures of *E. coli* K12 in the stationary phase. In the present work we study electric polarizability during culture growth with the aim of finding out how it is influenced by the physiological state of the cells. The electro-optical method of electric turbidimetry is used, which is based on the change in the optical density as a result of orientation of bacterial cells under the action of an applied electric field. Our results show that until the cell concentration increases exponentially, the polarizability and the cell size change synchronously, so that the polarizability is approximately a quadratic function of the average bacterial length. We explain this with dominance of the SChD component. However, that after the polarizability deceases twofold at insignificant length oscillations and the power of the function decreases to 1.5. The last result is interpreted as an increase in the MW component.

1. Introduction

The electric polarizability of bacteria is determined by their electrical and geometrical (shape and size) properties and the conductivity of the intracellular and extracellular medium [1]. There are known two basic types of surface electric polarizability – Maxwell–Wagner (MW) and surface charge-dependent (SChD) polarizability. MW polarizability is defined as accumulation of electric charge at the interface of two phases having different volume electric conductivities and dielectric permittivities when an electric field is applied [2]. Its value depends on the size and the shape of the particles, but its relaxation frequency is not dependent on their dimensions [3]. SChD (counterion) polarizability [4,5] is determined by the polarization of the electrical double layer (EDL) of the particles [6,7], mainly of its diffuse part [8], and both its value and its relaxation frequency are dependent on the size, shape, and surface charge density of the cells and the ionic strength of the medium.

Usually the most used factor of differentiation of the polarizability mechanisms is the frequency region of appearance and relaxation of every mechanism [1]. In the frequency range above a few kilohertz SChD components should decrease to complete disappearance according to the electro-optical literature, taking into account the large bacterial dimensions [1,4]. However, we have found unexpected dependence of the electric polarizability on the outer medium electrolyte concentration in the range 20 kHz–2 MHz, which cannot be explained by MW component exhibition [9]. The results show that the polarizability decreases with the increase in the ionic strength in the same way as DEL thickness does according to the Poisson–Boltzmann equation [10] (linearly with the decrease in the negative half power of the electrolyte concentration). Such behavior is characteristic of SChD polarizability, which has allowed us to conclude that this component has the main contribution to the external bacterial surface at the experimental frequencies.

In our previous work [9] we have used a significant difference between MW and SChD components—their opposite response to the medium ionic strength variation. In the present paper another distinguishing factor is considered—the different dependence of the both components on the bacterial dimensions [2,4,11]. So we could evaluate the character of the polarization mechanism by measuring its value at different cells' size.

It was shown in Ref. [12] that the polarizability of *Escherichia coli* K12 decreases with the growth time of the bacterial culture. A possible reason for this is the change in the size of the bacterial cells and the intracellular electrolyte concentration. The influence of the last parameter was investigated in our previous work [13] by an ethanol-induced increase of the membrane permeability [14]. So a possible way to study the contribution of MW and SChD components of the polarizability is the investigation of the change in the bacteria size and polarizability during the culture growth.

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2. Materials and methods

2.1. Materials

A bacterial culture of *E. coli* K12 was cultivated in standard Luria–Bertani (LB) medium supplemented by 1% glucose for 7.5 h at temperature 37 °C and pH 7.0 in an EloFerm incubator (Biotronix GmbH, Germany). The bacterial samples were taken from the incubator automatically every 10 min by an EloTrace 2.0 electro-optical device (Biotronix GmbH, Germany), starting from the beginning of the incubation.

2.2. Electric turbidimetry theory

The optical density (OD) A in the case of the bacterial suspension is determined by light scattering in all directions except rays falling into the photodetector. When an electric field is applied to the bacterial suspension, A is changed due to the cells' orientation. The absolute value of the electro-optical effect (EOE) is determined as $\Delta A = A_E - A$, where the index E is for the applied electric field. In the case of the steady-state EOE (ΔA_s), the orientation degree depends on the ratio of orientation energy γE^2 and the chaotic motion energy kT of bacteria with electric polarizability γ under the action of an electric field with intensity E, and the relative value of the EOE is

$$\Delta A_s/A = G(F) \cdot (\gamma E^2/15kT) \quad (1)$$

where $G(F)$ is an optical function which depends on the wavelength, the bacteria geometry (size and form) and the refractive indices of cells and the medium [11].

The EOE decay after the switching off of the electric field is defined by the rotational diffusion coefficient D, respectively, by the relaxation time $\tau = 1/6D$. The EOE decay of a monodisperse suspension is mono-exponential for particles with axial symmetry,

$$\Delta A_t = \Delta A_s \exp(-6Dt) = \Delta A_s \exp(-t/\tau) \quad (2)$$

where ΔA_t and ΔA_s are the values of EOE at the moment t and at steady-state, respectively [11].

2.3. Devices

The electro-optical measurements were carried out by an EloTrace 2.0 automatic device (Biotronix GmbH, Germany). The device provides continuous performance of the following basic operations with a period of $\geqslant$6 min: (1) taking an input suspension (with volume 1–2 ml) from the incubator, washing the cells by filtration, and suspending them in an aqueous medium with conductivity 5 μSm/cm; (2) dilution of the secondary suspension to 0.1 unit OD (per 10-mm optical path at 670 nm); (3) applying an electric field with preliminary chosen field strength 1–100 V/cm and frequency 20 kHz–20 MHz; (4) measurement of EOE as a difference $\Delta A = \Delta A^{\parallel} - \Delta A^{\perp}$ between effects for two orthogonally directed light beams (parallel and perpendicular to the electric field vector; the amplitude of ΔA is a sum of both components due to their opposite signs); and (5) calculation of the polarizability γ and the diffusion coefficient D according to Eqs. (1) and (2), respectively. The average bacterial size is calculated automatically from D using an embedded database for its dependence on the dimensions of cells with different shape and axial ratio.

The EloFerm incubator (Biotronix GmbH, Germany) records the values of OD, pH, and the amount of base added to maintain constant pH during the cell cultivation. The combined usage of the EloFerm and EloTrace 2.0 devices gives a unique opportunity to observe synchronously the growth stage of the culture and the assembly-averaged physical parameters of the bacterial cells in the suspension.

3. Results and discussion

3.1. Culture growth curve

In Fig. 1 is presented the dependence of the optical density (OD) on the time t of *E. coli* K12 culture growth in semilogarithmic coordinates. The curve shows four well-distinguished parts, known in the microbiology as lag phase (t = 0–40 minute in this case), exponential phase (40–170 min), postexponential phase (t = 170–370 min), and stationary phase ($t \geqslant 370$ min). It is known that in the lag phase the bacterial concentration does not change but the size increases, so OD increases insignificantly due to the cell mass increasing. After that the cell fission starts with a constant division rate (exponential phase). During the third phase (known as the postexponential stage [15]) the cell division rate decreases progressively because of the exhaustion of the minor components of the incubation medium. Although this phase is not described in the literature as a separate one, we have shown that it is characterized as a different physiological state (determined by the geometrical and electrical properties of the bacterial cells) [16]. In the stationary phase the cell concentration and physiology parameters are almost constant due to the depletion of the main nutritive components.

3.2. Time dependence of the electric polarizability

In Fig. 2 is shown the dependence of the ensemble-averaged electric polarizability γ of *E. coli* K12 on the growth time at three different frequencies (80, 190, and 400 kHz), measured sequentially for the sample taken at moment t. The higher value of γ at higher frequencies is in accordance with our previous results for alive *E. coli* K12, showing maximum at 600 kHz (Fig. 2 in Ref. [14]). The relative changes of γ are similar for each of the three time dependences; in particular, the ratio between the values of γ at two plateaus (at 120 and 420 min) is about 2 for each curve.

As far as there is no an essential difference in the time dependence of γ at the submegahertz frequencies used, we will discuss the polarizability variation at only one frequency in this range. Fig. 3 shows that γ increases twofold (at 120 kHz) until it reaches a plateau at 90 min, and after 170 min it decreases strongly and reaches a second plateau (at $t \geqslant 370$ min) with almost the same value of γ as that in the beginning of the cultivation (at t = 0).

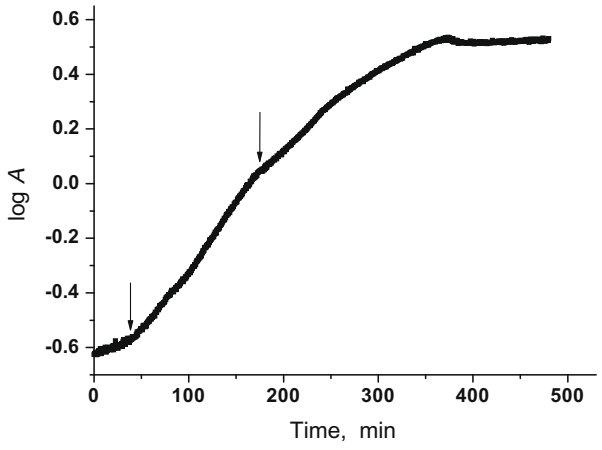

Fig. 1. Time dependence of the optical density A (at 620 nm and 10-mm optical path) for cultivation of bacteria *E. coli* K12 under standard conditions (medium LB supplemented with 1% glucose at pH 7.0 and 37 °C).