Colloids and Surfaces B: Biointerfaces 74 (2009) 23–27

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Influence of the medium electrolyte concentration on the electric polarizability of bacteria *Escherichia coli* in presence of ethanol

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ARTICLE INFO

Article history:
Received 10 February 2009
Received in revised form 12 June 2009
Accepted 13 June 2009
Available online 23 June 2009

Keywords:
Escherichia coli
Maxwell–Wagner polarizability
Charge dependent electric polarizability
Ethanol effect
Electrolyte effect
Electro-optics

ABSTRACT

The electric polarizability is an important parameter of bacteria, giving information about the electric properties of the cells. In our previous works [A.M. Zhivkov, A.Y. Gyurova, Colloids Surf. B: Biointerfaces 66 (2008) 201; A.Y. Gyurova, A.M. Zhivkov, Biophys. Chem., 139 (2009) 8; A.M. Zhivkov, A.Y. Gyurova, J. Phys. Chem. B, 113 (2009) 8375] we have applied an experimental approach to distinguish the contribution of the components of the two types of interface electric polarizability—surface charge dependent (ChD) and Maxwell–Wagner (MW) polarizability. It is based on electro-optical study of the separate influence of the outer and inner medium electrolyte concentration, which changes the external ChD and internal MW components of polarizability; the last effect is reached by the membrane permeability increase in low ethanol concentration. In the present work we investigate the behavior of electric polarizability of *Escherichia coli* K12 at increasing the outer KCl concentration in presence of 10 vol.% ethanol in order to check if the polarizability components change independently from one another. The conclusion is that the outer electrolyte concentration influence indirectly the internal MW component by the trans-membrane concentration gradient, but the polarizability components themselves change independently.

1. Introduction

The electric polarizability is an important parameter of bacteria, because it gives information about the electric properties of the cells. The methods used for investigation of electric parameters are electrophoresis [1,2] dielectric spectroscopy [3,4] dielectrophoresis [5] and electro-optics [6–9]. We apply the electro-optical (EO) method based on an orientation of disperse particles (bacteria in this case) in electric field; the degree of orientation is proportional on the polarizability. This method is much more sensitive than the others and allows measurements at low particle concentration.

Two main kinds of polarizability are known—volume and surface (interface) polarizability [6,10,7]. The first component is negligible in the case of biological cells. The interface polarizability is usually distinguished to charge dependent (ChD) and Maxwell–Wagner (MW) type. ChD polarizability is due to a movement of the counterions in the electric double layer (EDL) in presence of electric field. That is why it also is called EDL polarization or kilohertz polarization and usually is observed until some kilohertz [6,7,11]. It depends on the medium ionic strength, the size and the charge of the particles. MW polarizability appears at accumulation of ions on the interface between two phases under the action of electric field. This component depends on the electric conductivity and dielectric permittivity of both the phases; its relaxation is in the megahertz region and does not depend on the particles dimensions.

In the case of bacteria the cytoplasm and the lipid membrane are two phases with different electric parameters, so the cell has two MW components—external (EMW) and internal (IMW). The existence of two charged surfaces of bacteria cover leads to consider two ChD components—external (EChD) and internal (IChD). So, we take into account four components of the electric polarizability [12–14] like it was suggested for erythrocytes [5]. The considering of only two polarizability components is correct in the case of solid particles, but it has been applied for bacteria by now too [6,7,11].

In our articles regarding bacteria electric polarizability we have suggested an experimental way for distinguishing the types of surface polarizability by changing the outer and inner electrolyte concentration and with the frequency of the applied electric field [12–14]. Our approach is based on a principle difference between ChD and MW polarizability components—they have completely opposite behavior by varying the ionic strength: ChD polarizability decreases [6,7,11] and MW one increases [1,10] with the increase in the electrolyte concentration.

Our experiments have shown up the unexpected result that the increase in the outer ionic strength (from 0.1 mM to 1 mM) leads to significant decrease in the electric polarizability of bacteria *Escherichia coli* K12 at the frequency range 20 kHz–2 MHz [12]. It has been proved that the inner electrolyte concentration

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doi:10.1016/j.colsurfb.2009.06.017

is not changed by varying the outer one for the short time of the EO experiment. Additionally, according to the literature [1] EMW component does not exhibit at the low ionic strength of the outer medium, usual for the EO experiment. The results also show that the electric polarizability depends on the minus half power of the outer electrolyte concentration, as the EDL thickness does. So we have concluded that only EChD component has contribution on the outer bacteria surface and it is shown up to a few megahertz, contrary to the wide accepted conception.

To distinguish the contribution of IMW polarizability we have carried out the experiments in presence of ethanol [13]. The main ethanol effect was found to be increasing the membrane permeability, possibly by damaging the cell wall. The resulting ionic flow through the membrane gives opportunities to change the cytoplasm electrolyte concentration and therefore IChD and IMW components. The experiments have presented that the electric polarizability of *E. coli* K12 decreases linearly with the increase in the ethanol concentration when the bacteria are suspended in water–ethanol medium, i.e. with the decrease in the inner ionic strength. Furthermore, it has been shown that the increase in the ionic strength after incubation in 0.1 M KCl results in polarizability increase. We note that in the mentioned experiment the bacteria were firstly incubated in distilled water (which leads to a slow decreasing of the cytoplasm electrolyte concentration, which usual value is about 0.1 M [14]) and then in 0.15 M KCl, which causes cytoplasm concentration increasing (faster at the ethanol presence). The estimation of the inner electrolyte concentration showed that it is high enough and therefore IChD component practically does not exhibit. That let us to conclude that IMW component has the main contribution on the inner membrane surface at the frequency range mentioned above [14].

So, generally our experiments have shown that only EChD and IMW components contribute observably to the total bacteria polarizability at the conditions of the EO experiment. However, their contributions have been distinguished in separate experiments by changing the factors (outer and inner ionic strengths), which have been supposed to influence only one of them. The problem, investigated in the present work, is what the behavior of the bacteria electric polarizability would be, if we change the outer electrolyte concentration in presence of ethanol.

2. Materials and methods

2.1. Microorganisms, media and culture conditions

Bacteria culture of *E. coli* K12 was cultivated in standard medium Luria Bertani (LB) supplemented with 1% glucose for 7.5 h at temperature 37 °C and pH 7.0. The bacteria samples were taken from the culture medium, washed with distilled water on a millipore filter with size of the pores of 0.8 μm and suspended in 3% aqueous solution of formaldehyde. The cells were washed from the formaldehyde and suspended in distilled water just before the beginning of electro-optical measurements. The final suspension had electric conductivity of 5 μS/cm and optical density 0.1 at optical path 1 cm and wave length 670 nm.

2.2. Electro-optical method

When electric field is applied to the bacterial suspension, the optical density A is changed due to orientation of the cells. The value of the electro-optical effect (EOE) is determined as $\Delta A = A_E - A$, where the index E means presence of electric field. In steady-state EOE (ΔA_s) the degree of orientation depends on the ratio of the orientation energy γE^2 and the energy of chaotic motion kT of the cells:

$$\frac{\Delta A_s}{A} = G\left(\frac{\gamma E^2}{15kT}\right), \quad (1)$$

where γ is the bacteria electric polarizability in electric field with intensity E; G is an optical function, which depends on the size, form, and the refractive index of cells.

The EOE decay after switching off 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 system is monoexponential for bacterial cells with axial symmetry like *E. coli*:

$$\Delta A_t = \Delta A_s \exp(-6Dt) = \Delta A_s \exp\left(\frac{-t}{\tau}\right), \quad (2)$$

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

2.3. Electro-optical device

The main part of electro-optical measurements was executed by EloTrace-1.0, developed in Biotronix GmbH (Germany). The device automatically takes down the dependencies of the EOE on the frequency (20 kHz–20 MHz) and the intensity of the electric field (17–110 V/cm) at wavelength 670 nm and optical path 1 cm. It also calculates the polarizability and average size of bacteria cells. The rest of experiments were carried out by EloTrace-2.0 (Biotronix GmbH, Germany), which makes all the operations completely automatically. The device takes a bacteria sample of 1–2 ml from the incubator every ≥6 min, filtrates, washes, suspends and dilutes it until obtaining a suspension with a definite optical density and electric conductivity mentioned above. After that EloTrace-2.0 takes down and treats automatically the electro-optical data, so that the electric polarizability and average bacteria size with the growth time can be recorded.

All the experiments are carried out with a suspension of *E. coli* K12 fixed by formaldehyde at optical density 0.1 and electric field strength 78 V/cm.

3. Results and discussion

Figs. 1 and 2 present the frequency dependencies of the electric polarizability of *E. coli* K12 at different KCl concentration in absence and in presence of 10 vol.% ethanol. The increase in the electrolyte

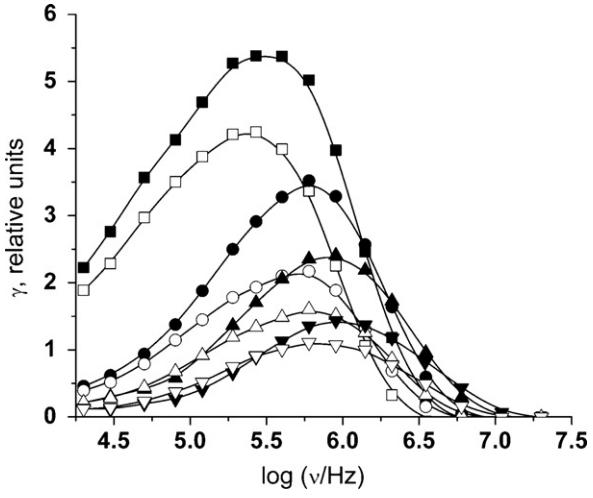

Fig. 1. Frequency dependencies of electric polarizability of *E. coli* K12: (■) without KCl and ethanol; (□)10 vol.% ethanol, without KCl; (●) 0.1 mM KCl, without ethanol; (○) 0.1 mM KCl, 10 vol.% ethanol; (▲) 0.25 mM KCl, without ethanol; (△) 0.25 mM KCl, 10 vol.% ethanol; (▼) 0.5 mM KCl, without ethanol; (▽) 0.5 mM KCl, 10 vol.% ethanol.