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EXPERIMENTAL ARTICLES

The Electro-Optical Investigation of Suspensions of *Escherichia coli* K-12 Cells Metabolizing Glucose, Lactose, and Galactose

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Abstract—The electro-optical characteristics of suspensions of *Escherichia coli* K-12 cells metabolizing glucose, lactose, and galactose were studied by measuring the suspension turbidity as a function of cell alignment in an orienting electric field whose frequency was varied from 10 kHz to 10 MHz. In a frequency range of 10 kHz to 1 MHz, the orientational spectra of *E. coli* K-12 cells grown on glucose and lactose considerably changed after their incubation in the presence of the sugars. These changes likely reflect alterations in the polarizability of the cells induced by sugar metabolism.

Key words: *Escherichia coli* K-12, glucose, metabolism, electrophysical properties of cell suspensions.

The electrophysical properties of bacterial cells have been described in a number of our recent publications [1–5]. The electro-optical analysis of *Brevibacterium* sp. 13PA and *Acinetobacter calcoaceticum* A-122 cells showed that their electrophysical properties undergo considerable changes during the preparatory metabolism of *p*-nitrophenol and acrylamide.

We continued these investigations using *Escherichia coli* K-12 cells, whose metabolism, including the metabolism of glucose, lactose, and other sugars, has been studied in depth [6, 7]. The relevant information available in the literature may provide a methodological basis for the analysis of correlation between the electrophysical properties of cells and the activity of their particular enzymatic systems.

The aim of the present work was to study the effect of glucose, lactose, and galactose metabolism on the electro-optical characteristics of *E. coli* K-12 cells.

MATERIALS AND METHODS

The electro-optical (EO) analysis of dispersed systems is based on their orientational alignment in static or varying electric fields, which results in optical anisotropic effects, such as birefringence, orientational dichroism, and an orientational turbidimetric effect [8]. The latter effect depends on the optical properties of the analyzed suspension and on the strength, frequency, and direction of the orienting field relative to the direction of a probing light beam. This effect can be evaluated by recording changes in the transmittance or absorbance (optical density) of the suspension.

The orienting field induces electric charges on suspended particles. The phenomenon of polarizability lies in the induction of electric charges at the interfaces between media with different permittivities. If the particles under study are microbial cells, electric charges are induced at the interfaces between the double electric layer surrounding a cell and the cell wall, between the cell wall and the cytoplasmic membrane, between the cytoplasmic membrane and the cytoplasm, and so on.

The values of the electric charges induced at the interfaces between different media are proportional to the electric field strength E and depend on the permittivities of these media. The induced electric charges are described by a tensor of the particle polarizability. For axially symmetrical particles, which well approximate many microbial cells, the polarizability tensor has two components, γ_a and γ_b, corresponding to the long particle's axis and the orthogonal direction, respectively [9]. The positive and negative charges induced on the surface and in the bulk of particles form effective dipoles, whose interaction with the external electric field aligns the particles.

The degree of particle alignment along and across the orienting electric field vector depends on the sign of the anisotropic polarizability tensor $\Delta\gamma = \gamma_a - \gamma_b$ and the value of the following parameter:

$$q = \Delta\gamma E^2/(2kT), \quad (1)$$

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where k is the Boltzmann constant and T is the absolute temperature. The value of this parameter is determined by the proportion between the energies of the orienting electric field and the Brownian motion of particles, which hinders their alignment. The range of the values $q \ll 1$ corresponds to the small degree of particle alignment.

Figure 1 presents oscillograms which were recorded using an unpolarized light beam with λ = 810 nm directed perpendicular to the orienting electric field vector with E = 100 V/cm [10]. The oscillograms represent a series of four successive pulses of an equal length (about 1 s) and different frequencies ω and reflect transient changes in the transparency of a cell suspension under the action of the orienting electric field.

An electro-optical signal has three regions. Region I corresponds to the stage of rapid increase in the transparency of a suspension of particles, region II corresponds to the quasistationary state of the suspended particles at a certain degree of their alignment with a weak dynamic modulation at a frequency two times higher than that of the orienting field, and region III is related to the relaxational transition of the suspension to a state with randomly oriented particles.

The orientational spectrum (OS) of a slightly ordered suspension can be described by the formula [12]:

$$\delta D(\omega) = (D_a - D_b)/D = \Delta\gamma(\omega)E^2F, \qquad (2)$$

where ω is the frequency of the orienting electric field, D_a and D_b are the optical densities of the suspension along (a) and across (b) the orienting field, D is the optical density of the suspension when their particles are fully disordered, and F is a coefficient allowing for the size and the refractive index of cells [8].

With an accuracy to a constant factor, the dependence δD coincides with the frequency variance $\Delta\gamma(\omega)$ of the anisotropic polarizability tensor. Depending on the orienting field frequency, the polarizability variance and, therefore, the orientational spectra of cells turn out to be dependent on their different structural elements. When the frequency $\Delta\gamma(\omega)$ is within tens of Hz, the OS of cells is mainly determined by the properties of the cell-surface biopolymers and associated high- and low-molecular-weight compounds, which affect the double electric layer formed at the interface between the cell and the surrounding medium. When this frequency comprises tens and hundreds of kHz, the OS is influenced by components of the cell wall and the cytoplasm. At frequencies of several to tens of MHz, the OS depends largely on the structural elements of various subcellular organelles. All this makes it possible to gain information on various physicochemical, physiological, and biochemical processes occurring on the surface and in the bulk of microbial cells.

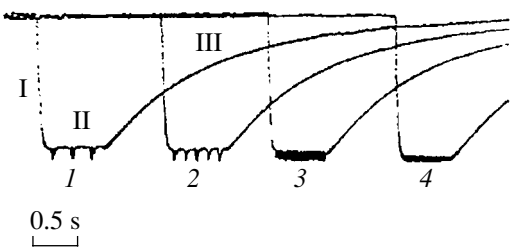

Fig. 1. Oscillograms of the electro-optical signals of *E. coli* K-12 cells recorded in the orienting electric fields with frequencies of (*1*) 2.5, (*2*) 7, (*3*) 14, and (*4*) 25 Hz. The oscillograms are reproduced from [10].

The orientational spectra of cells were recorded using an ELBIC electro-optical analyzer designed and manufactured at the State Research Center for Applied Microbiology. The analyzer presents orientational spectra in the form of the following frequency dependence:

$$\delta D_{rel} = \delta D/(E^2F\kappa) \approx A\Delta\gamma. \qquad (3)$$

Here the transmittance $\kappa = I/I_0$, where I and I_0 are the intensities of the passed and incident light beams, and A is a scaling constant that was introduced to make the read-out of the parameter δD_{rel} more convenient (in our experiments, the magnitude of this parameter was 10^2–10^3). At sufficiently low degrees of cell alignment, δD_{rel} is independent of the cell concentration, the intensity of the orienting electric field, and the extinction of light during its transmission through the suspension.

The strain *Escherichia coli* K-12 was obtained from the collection of microorganisms at the Institute of Biochemistry and Physiology of Plants and Microorganisms. The strain was grown aerobically at 30°C on a shaker (160 rpm) in two liquid media. Medium I contained (g/l) NaCl, 10; yeast extract, 5; and peptone, 5. Medium II contained (g/l) Na_2HPO_4 , 3; KH_2PO_4, 6; NaCl, 1.8; $CaCl_2$, 0.01; peptone, 6; and glucose (or lactose), 6.

After 24 h of cultivation, cells were collected by centrifugation at 2800 *g* for 5 min, washed, and resuspended in distilled water. Large cell aggregates were removed by centrifugation at 110 *g* rpm for 1 min, and the supernatant of the cell suspension with D_{670} = 0.4–0.45 was used to record orientational spectra. The suspension was preliminarily incubated with 10 g/l glucose, lactose, or galactose at 29°C for 30 min.

The orientational spectra were recorded at λ = 670 nm in a 1-ml electro-optical chamber at the following frequencies of the orienting electric field: 10, 52, 104, 502, 1000, 5020, and 10 000 kHz.

RESULTS AND DISCUSSION

E. coli is a facultative anaerobe capable of growing under both anaerobic and aerobic conditions [7]. Under aerobic conditions, growth substrates are partially oxi-

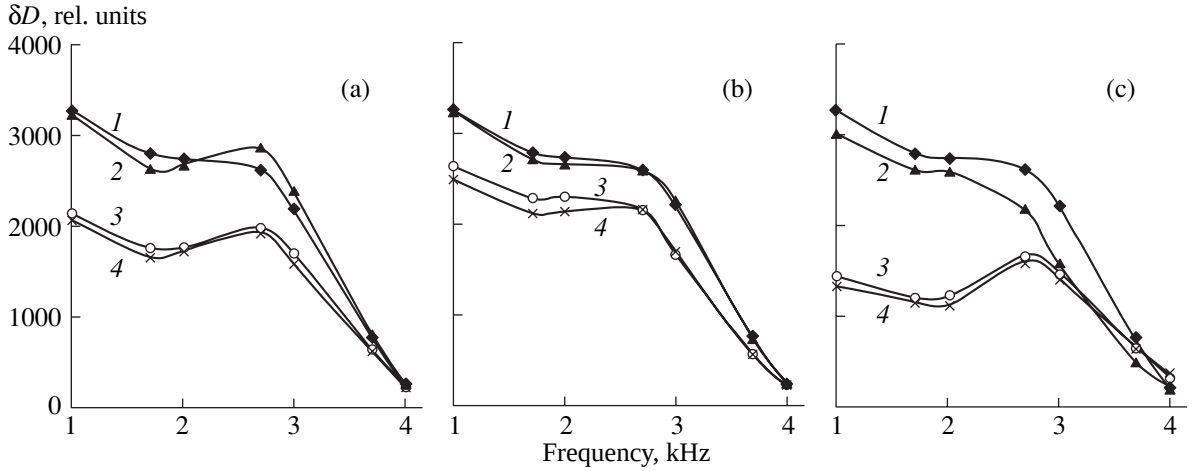

Fig. 2. The dynamics of the orientational spectra of *E. coli* K-12 cells incubated in the presence of (a) glucose, (b) lactose, and (c) galactose for (*2*) 0, (*3*) 15, and (*4*) 30 min. The control curves *1* represent the OS of the cells incubated in distilled water for 15 min.

dized to CO_2 and water with oxygen as the terminal electron acceptor. This process yields ATP, which is necessary for the biosynthesis of cellular constituents. In the case of glucose, about 50% of this substrate is oxidized to CO_2 and water with the formation of ATP, which is used to metabolize the other 50% of glucose [7].

Changes in the electrophysical parameters of bacterial cells metabolizing sugars may be related to the sugar transport into the cells, the enzymatic conversion of the sugars, or their nonspecific interaction with the cell surface. It should be noted that our earlier studies [1–5] showed that the preparatory metabolism of *p*-nitrophenol and acrylamide influences the electro-optical properties of *Brevibacterium* sp. 13PA and *Acinetobacter calcoaceticum* A-122 cells.

In the first set of experiments, *E. coli* K-12 cells were grown in a complex nutrient medium containing peptone and yeast extract as the sources of carbon and energy. The OS of cells incubated at 29°C in the presence of 10 g/l glucose, lactose, or galactose were recorded after 0, 15, and 30 min of incubation (Fig. 2). As can be seen from this figure, the OS of cells exhib-

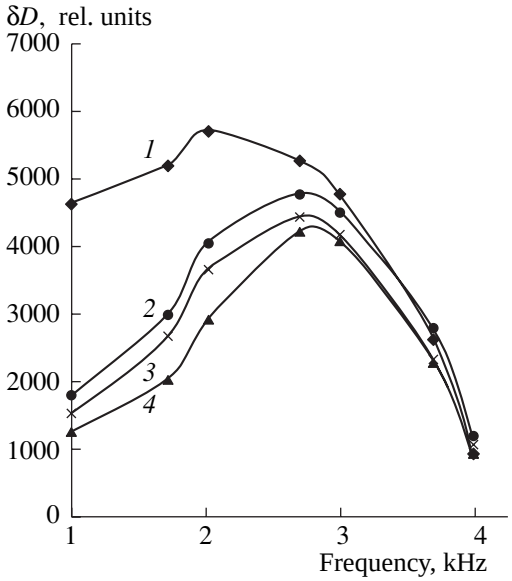

Fig. 3. The orientational spectra of glucose-grown *E. coli* K-12 cells incubated in the presence of (*2*) lactose, (*3*) galactose, and (*4*) glucose for 15 min. The control curve *1* represents the OS of the cells incubated in distilled water for 15 min.

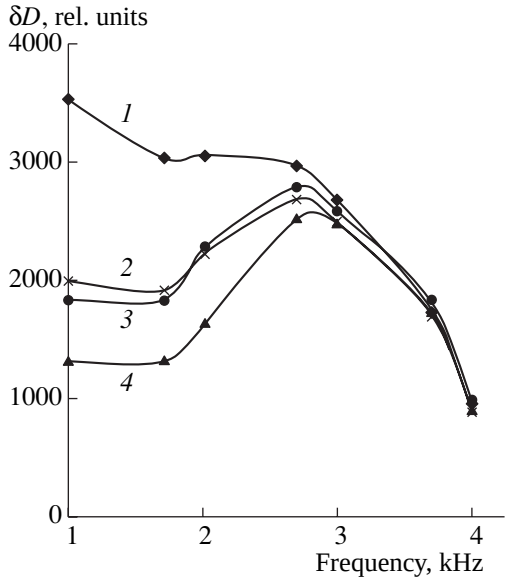

Fig. 4. The orientational spectra of lactose-grown *E. coli* K-12 cells incubated in the presence of (*2*) galactose, (*3*) lactose, and (*4*) glucose for 15 min. The control curve *1* represents the OS of the cells incubated in distilled water for 15 min.

ited notable changes not immediately after the addition of the sugars but 15 min afterwards. Taking into account the high rate of the nonspecific sorption of low-molecular-weight compounds on the surface of colloid particles [13], we can suggest that the observed changes in the OS of *E. coli* K-12 cells are associated with the metabolism of the sugars rather than with their nonspecific sorption on the cell surface. It should be noted that noticeable changes in the OS of the cells were observed only at the orienting electric field frequencies within a range of 10 kHz to 1 MHz. Similar results were obtained with *E. coli* K-12 cells grown on nutrient agar.

In the second set of experiments, *E. coli* K-12 cells were grown in media with peptone and glucose (or lactose). The OS of glucose- and lactose-grown cells are presented in Figs. 3 and 4, respectively. The incubation of *E. coli* K-12 cells with glucose led to considerable changes in their orientational spectra, irrespective of the composition of the medium in which these cells were grown. This may be related to the fact that the glucose metabolism enzymes of *E. coli* K-12 are constitutive. The metabolism of glucose in this bacterium involves several stages: (1) glucose transport into the cell, (2) the conversion of glucose-6-phosphate into pyruvate via the Embden–Meyerhof–Parnas pathway, (3) the oxidative decarboxylation of pyruvate to acetyl-CoA, and (4) the oxidation of acetyl-CoA in the tricarboxylic acid cycle [7]. For the glucose-grown *E. coli* K-12 cells to be able to utilize lactose, some specific enzymes of lactose metabolism should be induced. This may change the electric properties of cellular structures and, hence, affect the OS of the cells.

The incubation of the glucose- and lactose-grown cells in the presence of lactose or galactose exerted similar effect on the OS of the cells. Since lactose in bacterial cells is splitted to glucose and galactose [7], lactose must induce the enzymatic systems that are responsible for the metabolism of both glucose and galactose. The observed changes in the OS of cells incubated in the presence of lactose may be related to the activation of enzymes involved in galactose metabolism.

Thus, the metabolism of sugars influences the electrophysical properties of bacterial cells. This finding adds up to our earlier observation that the metabolism of toxic compounds affects the orientational spectra of microbial cells [1–5]. Investigations along this line of research should be continued toward the evaluation of the effect of particular enzymatic systems and individual enzymes on the electrophysical characteristics of cells. These studies may employ specific inhibitors and mutant strains.

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