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EXPERIMENTAL ARTICLES

The Effect of Ampicillin on the Electrophysical Properties of *Escherichia coli* Cells

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Abstract—The study of the effect of ampicillin on the electrophysical properties of *Escherichia coli* cells showed that this antibiotic influences the orientational spectra (OSs) of the ampicillin-susceptible *E. coli* strains K-12 and XL-1 within the frequency range 10–1000 kHz of the orienting electric field and does not affect the OSs of the ampicillin-resistant strains K-12(pUC-18) and XL-1(pHEN1). The change in the electrooptical signal of the ampicillin-susceptible cells was maximum at an ampicillin concentration of 50 μg/ml and did not depend on the exposure time. The conclusion is drawn that changes in the OSs of cells can be used to evaluate their resistance to ampicillin.

Key words: *Escherichia coli*, orientational spectra, ampicillin, plasmids.

The resistance of microorganisms to antibiotics is an important theoretical and practical problem.

Ampicillin is a broad-spectrum β-lactam antibiotic, which is produced by the acetylation of 6-aminopenicillic acid with aminophenylacetic acid. The action of ampicillin on susceptible microorganisms (including *Escherichia coli*) is accompanied by both morphological (cell elongation, bending, swelling, aggregation, and partial or complete lysis of the cell wall) and metabolic changes [1]. The mechanism of action of β-lactam antibiotics lies in their interfering with the synthesis of the bacterial cell wall, specifically, its major component peptidoglycan. The inhibitory action of β-lactams on the transpeptidase reaction results in an impairment of the formation of peptide bridges between parallel glycan filaments [1, 2].

The morphological alterations and the altered synthesis of the cell wall must affect the electrophysical properties of microbial cells, including the electrooptical parameters of cell suspensions recorded in electric fields. This phenomenon can be used to evaluate the resistance of cells to particular antibiotics.

The aim of this work was to study the effect of ampicillin on the electrooptical parameters of *E. coli* cells differing in their resistance to this antibiotic.

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MATERIALS AND METHODS

Experiments were carried out with the *Escherichia coli* strains XL-1, K-12, and K-12(pUC-18), which were obtained from the collection at the Institute of Biochemistry and Physiology of Plants and Microorganisms. The strains were grown aerobically at 30°C on a shaker (160 rpm) in a liquid nutrient medium containing (g/l) NaCl, 10; yeast extract, 5; and peptone, 5. *E. coli* cells grown in this medium for 1 day were harvested and used for electrooptical measurements.

Cell preparation. Before analysis, cells were washed three times by centrifugation at 2800 *g* for 5 min and resuspended in a small volume of distilled water with a conductivity of 1.8 μS/cm. The cell suspension was centrifuged at 110 *g* for 1 min to remove cell clumps, and the supernatant was used for electrooptical measurements. Preliminarily, the optical density of the supernatant measured at 670 nm (D_{670}) was adjusted to 0.4–0.42 for each of the bacterial strains used.

The orientational spectra of cells were recorded at 670 nm as described earlier [3] by using an ELBIC electrooptical analyzer designed and manufactured at the State Research Center for Applied Microbiology. The orienting electric field had discrete frequencies of 10, 52, 104, 502, 1000, 5020, and 10000 kHz. The orientational spectrum of a cell suspension was presented as a frequency dependence of the difference of the optical densities of the cell suspension measured along and across the orienting field vector. The optical density difference was normalized to the optical density of the cell

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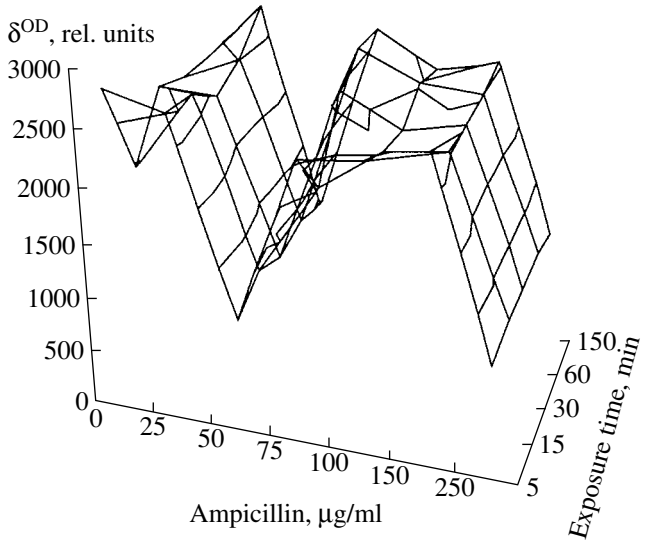

Fig. 1. The effect of different concentrations of ampicillin (25, 50, 75, 100, 150, and 250 μg/ml) on the electrooptical signal of *E. coli* K-12 cells recorded in an orienting electric field with a frequency of 52 kHz.

suspension measured at a random cell orientation. There are grounds to believe that, at certain wavelengths of the light beam and the amplitude of the orienting electric field, the orientational spectrum is mainly determined by the frequency dependence of the cell anisotropy [4–6].

Cell transfection (which occurs with the involvement of bacterial F pili) was carried out with the phagemid plasmid pHEN1. Cells of the strain *E. coli* XL-1 Blue *recA1 endA1 gyrA96 thi*-1 *hsdR17 supE44 relA1 lac*[F'*proABlacI*^{q}ZΔM15Tn10(Tet^R)] (Stratagene), which was obtained from A. Laman (Branch of the Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Pushchino, Moscow oblast), were transferred from a single colony grown on a plate with 2× YT agar medium containing 125 μg/ml tetracycline to 2 ml of a liquid 2×YT medium, which contained (g/l) NaCl, 5; yeast extract (Fluka, Switzerland), 10; and an amount of tryptone (Fluka). The inoculated medium was cultivated overnight at 37°C. A one-tenth portion of the overnight culture was transferred to fresh medium and cultivated to the early exponential growth phase (D_{600} = 0.5–0.6). At this moment, the aeration of the culture was suspended for 30–40 min to restore F pili. Then the culture was mixed with a suspension of phage particles, whose number exceeded the number of bacterial cells by 20 times. The mixture was incubated at 37°C under stationary conditions (to induce the sorption of phage particles on the pilus surface). The cells were precipitated by centrifugation at 2000 *g* for 10 min and resuspended in a fresh 2× YT medium containing 100 μg/ml ampicillin. The phage-infected bacterial cells were incubated overnight at 37°C on a shaker (180 rpm) with aeration and used for electrooptical measurements.

RESULTS AND DISCUSSION

The biological activity of β-lactam antibiotics is mainly determined by their ability to interact with the cell surface and to alter the barrier properties of the cytoplasmic membrane. Ampicillin was chosen for our experiments with *E. coli* cells because of its activity against a wide range of gram-negative bacteria.

The ability of ampicillin to affect the electrophysical properties of *E. coli* cells was studied by using the ampicillin-susceptible strain K-12, the ampicillin-resistant strain K-12(pUC-18), and the ampicillin-susceptible strain XL-1 transfected with the ampicillin-resistant phagemid plasmid pHEN1.

Six aliquots of a suspension of *E. coli* K-12 cells were supplemented with ampicillin to final concentrations of 25, 50, 75, 100, 150, and 250 μg/ml; incubated at 30°C; and subjected to electrooptical measurements. Substantial changes in the orientational spectra (OSs) of the ampicillin-treated *E. coli* K-12 cells were observed at the first five frequencies (10, 52, 104, 502, and 1000 kHz) of the orienting electric field. For the sake of simplicity, however, Figs. 1–5 present only the experimental data obtained for one field frequency (52 kHz).

Figure 1 shows the effect of different concentrations of ampicillin on the electrooptical signal of bacterial cells. As is evident from this figure, the decrease in the electrooptical signal was maximal at an ampicillin concentration of 50 μg/ml.

It is known that the effect of an antibiotic on bacterial cells can be either bacteriostatic or bactericidal, depending on its concentration and the mechanism of action. The bactericidal concentrations of ampicillin are two to ten times higher than its bacteriostatic concentrations [1]. The bacteriostatic effect of β-lactams consists in that susceptible bacterial cells exposed to these antibiotics lose their ability to form septa during fission, with the result that the undivided cells transform into filaments. At bactericidal concentrations, β-lactams cause a deformation of the cell wall and then its lysis. The lysis of *E. coli* K-12 cells exposed to high ampicillin concentrations occurs earlier than the formation of filamentous cells. The observed decrease in the electrooptical signal of these cells induced by 50 μg/ml ampicillin was likely to be due to a deformation of the cell wall during cell fission. Consequently, a concentration of ampicillin equal to 50 μg/ml can be considered bactericidal to *E. coli* K-12 cells.

The study of the effect of different exposure times (5, 15, 30, 60, and 150 min) on the electrooptical signal of *E. coli* K-12 cells induced by 50 μg/ml ampicillin showed that, as soon as after 5-min incubation, ampicillin induced a noticeable decrease in the electrooptical signal of these cells (Fig. 2). This is in agreement with the fact that antibiotics adsorb on the cell wall of bacteria as rapidly as within 2 min [7]. The decrease in the electrooptical signal of cells was at a maximum after

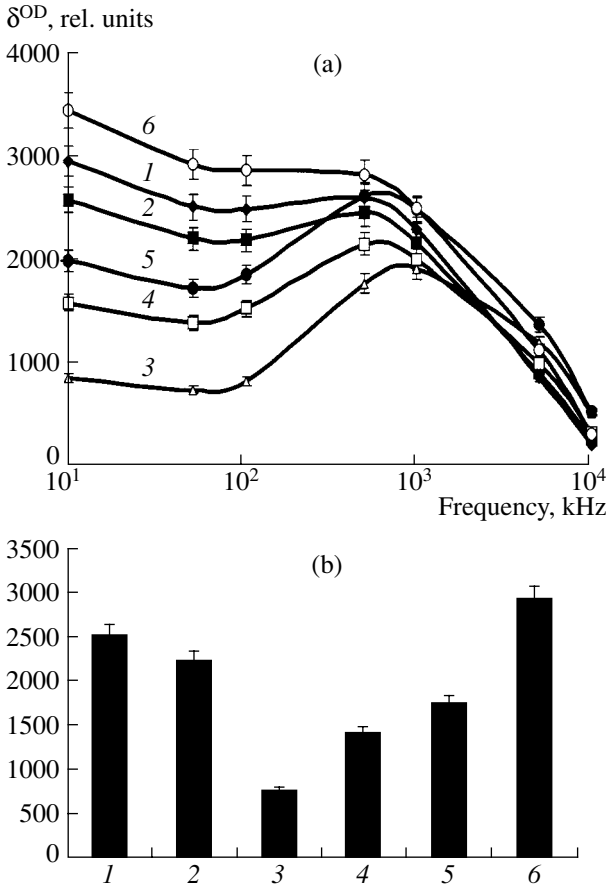

Fig. 2. (a) The orientational spectra recorded at 52 kHz and (b) the electrooptical signals of the ampicillin-susceptible *E. coli* K-12 cells incubated in the presence of 50 μg/ml ampicillin for (*1*) 0, (*2*) 5, (*3*) 15, (*4*) 30, (*5*) 60, and (*6*) 150 min.

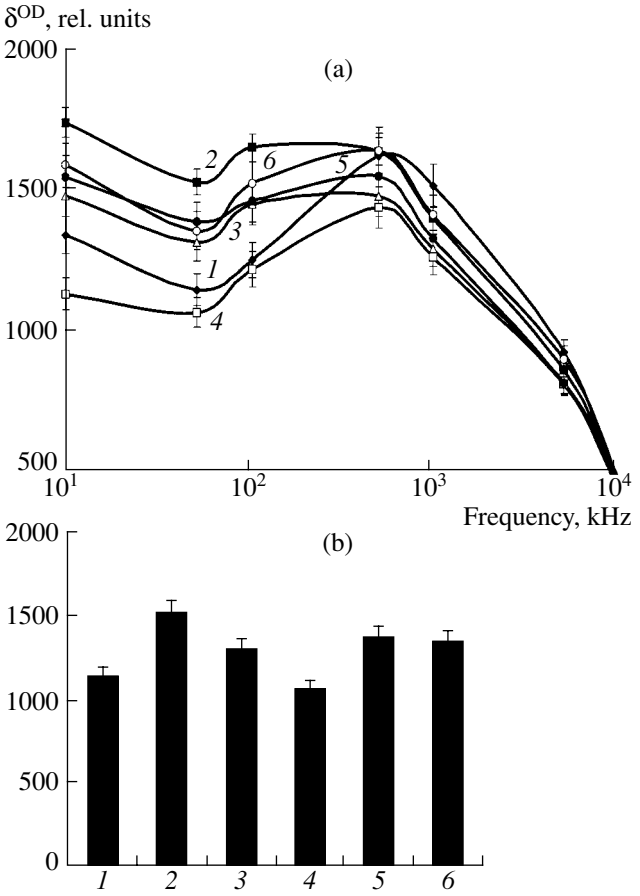

Fig. 3. (a) The orientational spectra recorded at 52 kHz and (b) the electrooptical signals of the ampicillin-resistant *E. coli* K-12(pUC-18) cells incubated in the presence of 50 μg/ml ampicillin for (*1*) 0, (*2*) 5, (*3*) 15, (*4*) 30, (*5*) 60, and (*6*) 150 min.

15-min exposure to ampicillin. Within this time period, the antibiotic is likely to cause a deformation of the cell wall. After 30-min incubation with ampicillin (when the activity of this antibiotic is at a maximum [7]), the electrooptical signal of *E. coli* K-12 cells increased.

Similar experiments carried out with the ampicillin-resistant strain *E. coli* K-12(pUC-18) showed that ampicillin virtually did not affect the electrooptical signal of the cells of this strain, except that there was a slight increase in this signal after 5-min exposure (Fig. 3). The absence of a notable effect of ampicillin on the electrooptical signal of cells can be considered as an indication of their ampicillin resistance. The small increase in the electrooptical signal of cells observed after 5-min exposure to ampicillin may be related to the adsorption of this antibiotic on the cell surface since it is known that ampicillin adsorbs at a high rate on both resistant and susceptible bacterial cells, the process of adsorption being independent of the concentration of ampicillin in the medium [7].

The suggestion that ampicillin affects the electrooptical signal of susceptible bacterial cells and does not change the electrooptical signal of resistant bacterial cells was proved by experiments with another ampicillin-susceptible *E. coli* strain, XL-1. The incubation of the cells of this strain with 50 μg/ml ampicillin for 5, 15, 30, 60, and 150 min was accompanied by an increase in their electrooptical signal at all the incubation times (Fig. 4). Then these cells (which carry an F episome and have F pili and, consequently, can be infected by phages [8]) were subjected to transfection with plasmid pHEN1 bearing the ampicillin resistance gene. This plasmid is based on the M13K07 phage genome; can replicate as an ordinary plasmid; and, due to the presence of the *ori* replicon of the phage, is able to form phagemid particles and infect bacterial cells with an F episome [9]. After transfection, the XL-1 cells with plasmid pHEN1 were incubated with 50 μg/ml ampi-

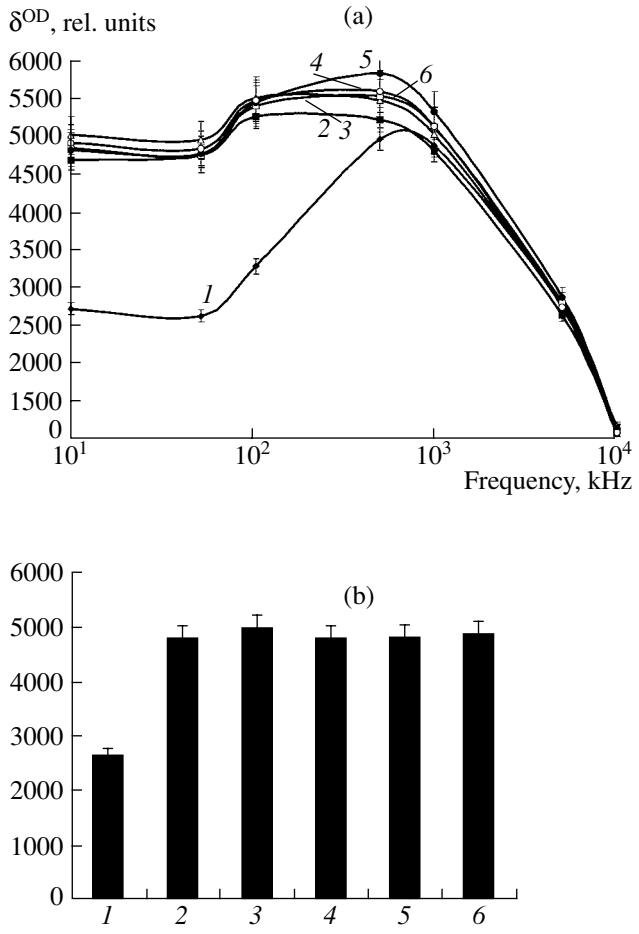

Fig. 4. (a) The orientational spectra recorded at 52 kHz and (b) the electrooptical signals of the ampicillin-susceptible *E. coli* XL-1 cells incubated in the presence of 50 μg/ml ampicillin for (*1*) 0, (*2*) 5, (*3*) 15, (*4*) 30, (*5*) 60, and (*6*) 150 min.

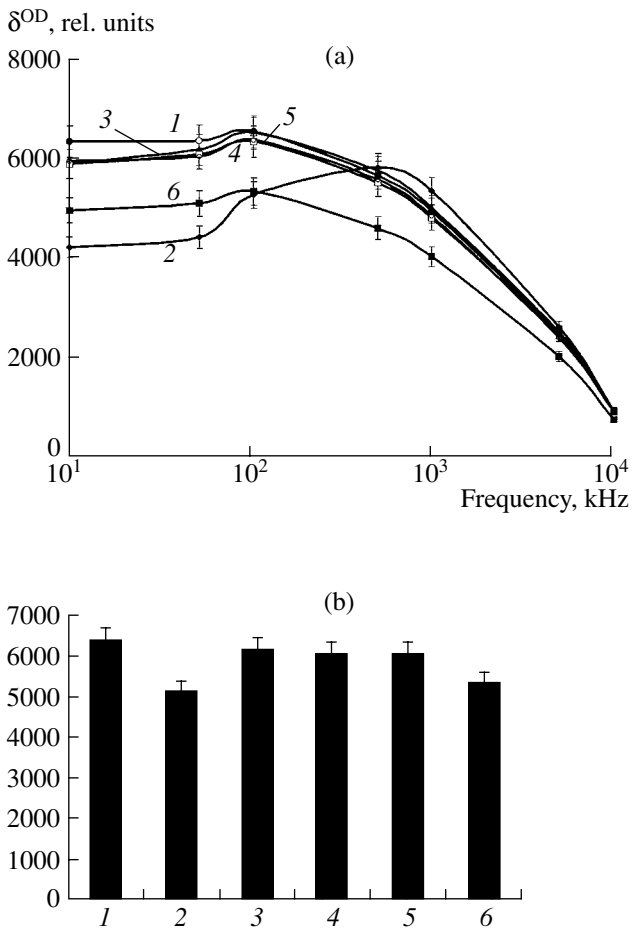

Fig. 5. (a) The orientational spectra recorded at 52 kHz and (b) the electrooptical signals of the ampicillin-resistant *E. coli* XL-1(pHEN1) cells incubated in the presence of 50 μg/ml ampicillin for (*1*) 0, (*2*) 5, (*3*) 15, (*4*) 30, (*5*) 60, and (*6*) 150 min.

cillin for 5, 15, 30, 60, and 150 min and then analyzed for the value of their electrooptical signal, which turned out to be maximum after a 5-min exposure to the antibiotic (Fig. 5). At longer incubation times, the electrooptical signal of cells did not change, suggesting that these cells became ampicillin-resistant.

Thus, ampicillin changes the electrooptical signal of the ampicillin-susceptible *E. coli* K-12 and XL-1 cells but does not affect the electrooptical signal of the ampicillin-resistant cells of *E. coli* strains K-12(pUC-18) and XL-1(pHEN1). The electrooptical signal of bacterial cells can be used to test their resistance to ampicillin and other antibiotics.

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