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EXPERIMENTAL ARTICLES

Dynamics of the Changes of Electrophysical Properties of *Azospirillum brasilense* Sp7 Cells at their Binding with Wheat Germ Agglutinin

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Abstract—The electrooptical properties of *Azospirillum brasilense* Sp7 cell suspensions, have been studied at a specific interaction with wheat germ agglutinin (WGA), using the dependences between the changes of optical densities of cell suspensions at the electric orientation of cells and the orienting field frequencies of 740, 1000, 1450, 2000, and 2800 kHz. It was shown that the electrooptical (EO) properties of cell suspensions changed at the interaction of *A. brasilense* Sp7 cells with WGA and that the EO signal value changed irrespective of the cultivation conditions. At the same time, the dynamics of the changes of the EO properties of microbial suspensions was different for microbial cells grown under different conditions. It may be evidence of the differences in the cell surface properties of microbial cells, and of the dependence, between bacterial response to lectin and growth conditions. The possibility of using the EO analysis of bacterial suspensions for the study of the high-specific binding of polypeptide molecular signals with the bacterial target cells and for assessment of the dynamics of this process has been demonstrated.

Key words: *Azospirillum brasilense* Sp7, electrophysical properties, wheat germ agglutinin

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One of the striking achievements of modern molecular microbiology is recognition of the fact that many bacteria are able to perceive proteins, which are present in the habitat in pico- and nanomolar concentrations, as molecular signals, and they respond by changing the program of population development. The cases when bacteria respond in this way to a protein of a host macroorganism have been described [1–5]; the synthesis of such signal proteins by bacteria is known as well. In the latter case, some cells of a bacterial population produce a physiologically active protein, while other cells are probably target cells, i.e. receive a signal and respond to it [6–8].

The most convenient model for the study of initial molecular events of the interaction of a signal protein with a bacterial target cell is, in our opinion, the interaction of the lectin of wheat germ agglutinin (WGA) with the cells of *Azospirillum brasilense* or *A. lipoferum*. Azospirilla, being natural symbionts of wheat, are able to react to WGA (a 36-kDa protein excreted by the plant in the places of wheat root colonization by azospirilla) by intensified nitrogen fixation [1, 9], stimulation of the transport of ammonium (the nitrogen fixation product) outside the bacterial cell [1], and intensified production of phytohormone IAA by the bacterium [4].

It has been established that the WGA receptor is a surface glycoprotein of azospirilla; in *A. lipoferum* Br17, its molecular weight is 32 kDa [9]. It is also known that the binding of lectin with the receptor glycoprotein is highly specific and conditioned by WGA binding with receptor regions, containing the dimer fragments of *N*-acetyl-D-glucosamine [1, 4, 9]. The data accumulated by now clearly demonstrates that the interaction of WGA with azospirilla cells is strictly regulated, although the details of this regulation are still unknown.

In view of the above, the objectives of this research were to study the dynamics of WGA binding with *A. brasilense* Sp7 cells grown under nitrogen fixation and microaerobically under conditions of ammonium assimilation. In each of these two cases, the cells are competent to the given signal protein [4, 9]. Since the approaches used by other authors provided no information about the dynamics of WGA binding with azospirilla cells, we used a new approach: registration of electrooptical changes of cell suspensions in response to the effect of a stimulus.

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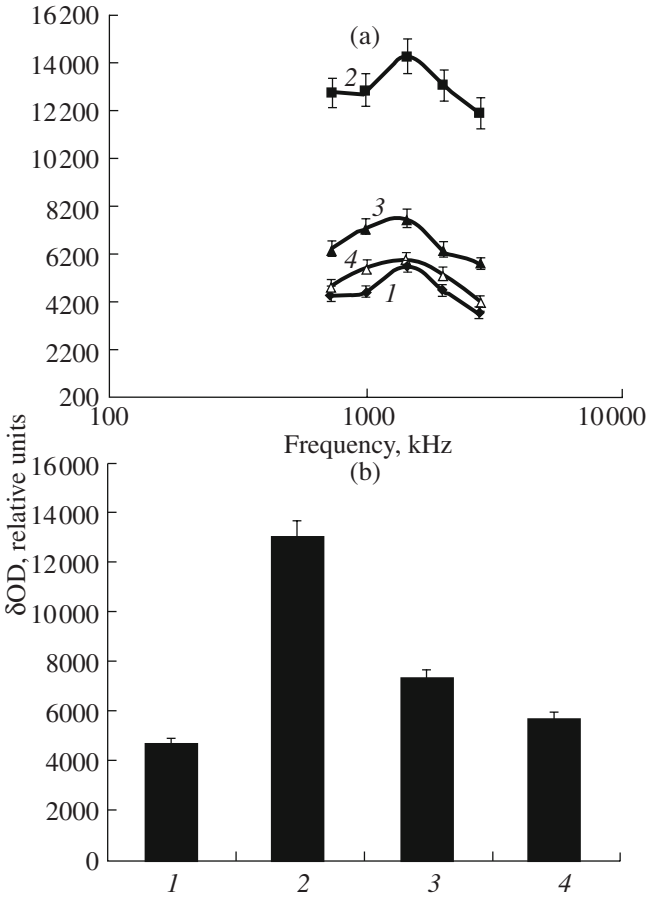

Fig. 1. Orientation spectra (a) and the change of relative values at the orienting field frequency of 1000 kHz (b) of *A. brasilense* Sp7 cells grown under nitrogen fixation conditions with addition of WGA: *1*, control (without WGA); *2*, after 30 min; *3*, after 60 min; *4*, after 90 min.

MATERIALS AND METHODS

Bacteria and cultivation conditions. The bacterium *A. brasilense* Sp7 was obtained from the collection of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Saratov).

A. brasilense Sp7 was maintained on a solid nutrient LB medium. The bacteria were grown in a synthetic malate–salt (SMS) medium [10] of the following composition (g/l): K_2HPO_4 – 3; KH_2PO_4, 2; NaCl, 0.1; $MgSO_4 \cdot 7H_2O$ – 0.31; $CaCl_2$ – 0.02; $FeSO_4 \cdot 7H_2O$ – 0.02; $MnSO_4 \cdot 7H_2O$ – 0.1; $Na_2MoO_4 \cdot 2H_2O$, 0.002; malic acid, 3.76; NaOH, 2.24; NH_4Cl, 3.0; yeast extract, 0.1; pH 6.8.

The inoculum was grown in 200-ml conical flasks (with 40 ml of the medium) for 18 h in an SMS medium, in a shaker at 32°C; the density of the grown culture was 10^9 cells/ml. The starting density of the cultures in all experiments was 10^7 cells/ml.

For the measurement of the electrooptical parameters of microbial suspensions, the cultures were grown in conical flasks in a liquid SMS medium for 18 h under varied conditions of nitrogen supply. The conditions of nitrogen fixation were created by excluding ammonium from the medium and growing the culture without agitation. The conditions of ammonium assimilation were identical to those for nitrogen fixation, with the only exception being, the medium contained ammonium.

Preparation of the cells for analysis. Prior to electrooptical analysis, the cells were washed three times with distilled water for 5 min, centrifuged at 2800 *g*, and resuspended in a small amount of distilled water (electroconductivity of 1.8 mk Cm). For elimination of conglomerates, the suspension was centrifuged again at 110 *g* for 1 min; the suspension in the supernatant was used. Then, optical density (D_{670}) of the suspension was brought to 0.4–0.42.

Measurement of the orientation spectra of the cells. Orientation spectra of the cells were measured in an ELUS electrooptical analyzer designed at the State Research Center of Applied Microbiology (Obolensk, Moscow oblast) as described in [11]. The electrooptical parameters of the cells were measured under the following conditions: electric field strength, 17 V/cm; the time of exposure to electric field, 16 sec; measurements were performed at the frequencies of 740, 1000, 1450, 2000, and 2800 kHz; cell number was 4.2×10^7 cells/ml.

The tested substances (WGA, *N*-acetyl-D-glucosamine, or WGA pre-incubated for 30 min with *N*-acetyl-D-glucosamine) were added to bacterial suspensions in the analyzer cuvette immediately before the measurements. Proteins were introduced on the basis of 1 μg WGA per every 10^7 azospirilla cells; *N*-acetyl-D-glucosamine was added to the final concentration of 1 μg/ml. WGA was preincubated with *N*-acetyl-D-glucosamine for 30 min by mixing the solutions of the lectin and its hapten (1 μg/ml of each); then the mixture was added to the bacterial suspension and the measurements were performed.

RESULTS

The series of our previous research into the electrophysical properties of microorganisms, using bacterial cells of different taxonomic affiliation and various acting agents (xenobiotics, antibodies, phages, and antibiotics), clearly demonstrated a common pattern: in the absence of a specific or nonspecific interaction of an acting agent with bacterial cells, the electrooptical (EO) properties of cell suspensions were unchanged after its addition. On the contrary, a specific interaction of a substance and cells resulted in a marked change of the value of the EO properties of cell suspensions [11–13].

The EO signal of *A. brasilense* Sp7 cells grown under nitrogen fixation conditions is shown in Fig. 1 (curve *1*). This curve presents the dependence of changes in the optical density of the azospirilla cell suspensions on the orienting field frequency in the range of 740–2800 kHz. Addition of WGA to the culture

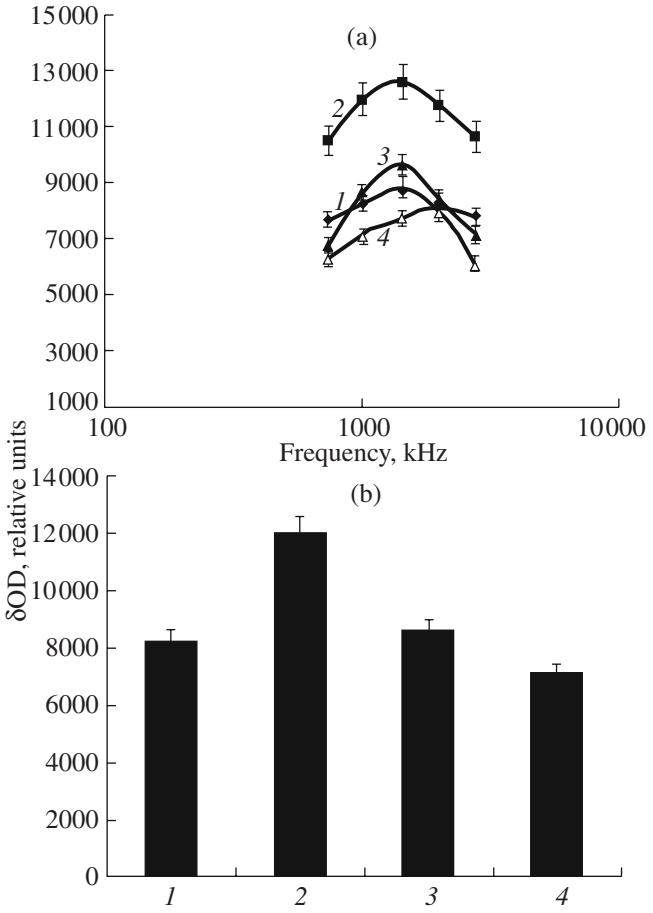

Fig. 2. Orientation spectra (a) and the change of relative values at the orienting field frequency of 1000 kHz (b) of *A. brasilense* Sp7 cells grown under ammonium assimilation conditions with addition of WGA: *1*, control (without WGA); *2*, after 30 min; *3*, after 60 min; *4*, after 90 min.

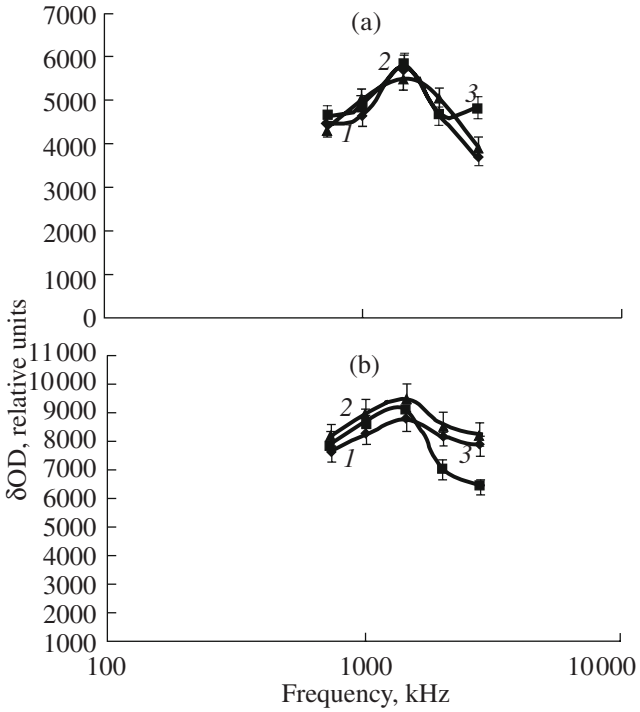

Fig. 3. Orientation spectra of the suspension of *A. brasilense* Sp7 cells grown under nitrogen fixation (a) and under ammonium assimilation conditions (b) with addition of *N*-acetyl-D-glucosamine and WGA pre-incubated with *N*-acetyl-*D*-glucosamine: *1*, control (without lectin); *2*, with *N*-acetyl-D-glucosamine; *3*, with WGA pre-incubated with *N*-acetyl-D-glucosamine.

resulted in an abrupt increase of the EO signal value (Fig. 1, curve *2*) measured 30 min after the onset of the stimulus action. After 60 min, the measured indices abruptly decreased; after 90 min, the value of the EO signal of the cell culture with lectin almost did not differ from the control (Fig. 1, curves *3* and *4*, respectively). The dynamics of the changes of the EO parameters caused by the presence of lectin in the medium for the frequency of 1000 kHz is shown in Figure 1b.

The culture of *A. brasilense* Sp7 grown in the ammonium assimilation conditions reacted to WGA in the same way as the culture grown under nitrogen fixation. Like in the first case, a marked intensification of the EO signal value was observed 30 min after the WGA addition (Fig. 2, curves *1* and *2*). It is important to note that the EO parameters of the cells with WGA, in the case of azospirilla cells grown under ammonium assimilation, became almost indistinguishable from the control after 60 min (Fig. 2, curve *3*) rather than after 90 min as in the first case (Fig. 1a, curve *4*). The second difference was the lower value of the EO signal of microaerobically grown azospirilla cells after 90 min of incubation with lectin at 740, 1000, 1450, 2000 and 2800 kHz as compared with the control (Fig. 2, curve *4*). As it concerns the frequency of 1000 kHz, the electrooptical signal returned to the control value after 60 min of azospirilla incubation with lectin and remained unchanged until the end of the experiment.

The following control experiments were performed, to verify the suggestion that the observed changes of the EO parameters of the cell suspensions are determined by specific binding of WGA with *N*-acetyl-D-glucosamine-containing fragments of the receptor on the bacterial surface. With this purpose, WGA was added to the suspension of the cells, which carbohydrate-binding sites were blocked with *N*-acetyl-D-glucosamine. Besides, the EO parameters were registered in cell suspension with addition of *N*-acetyl-D-glucosamine. In both cases (Fig. 3), the obtained results were not different from the control; this finding demonstrates that the change of the EO parameters of azospirilla cell suspension observed under the effect of WGA is caused by the binding of lectin molecules with *N*-acetyl-D-glucosamine-containing receptors exposed on the surface of bacterial cells.

DISCUSSION

The binding of WGA with the cells of *A. brasilense* and *A. lipoferum* is a well-established fact [14, 15]. Nevertheless, the approaches used in the previous studies, i.e. spectroturbidimetrical analysis [16], fluorescence microscopy [9, 15], flow cytofluorimetry [9, 14], and dot-blot analysis with colloidal gold [17], gave no possibility for studying the dynamics of the lectin interaction with azospirilla cells. The proposed method of registration of the changes of the EO parameters of cell suspensions, which result from the binding of signal protein with bacterial cells, makes it possible to monitor the dynamics of this process.

Different WGA-binding abilities of nitrogen-fixing and ammonium-assimilating populations of azospirilla accounted for different amplitudes of changes of their EO properties under the effect of WGA. As can be seen from the data presented above, a 30-min incubation with lectin resulted in an approximately threefold increase of the EO signal at a frequency of 1000 kHz under nitrogen fixation, whereas its increase in the cells grown in the presence of ammonium (ammonium assimilation conditions) was less pronounced (Figs. 1 and 2).

So far, it is unclear which of the surface structures of azospirilla bind WGA. At present, the generally accepted point of view is that proteins or glycoproteins exposed on the cell surface act as the receptors for proteins and hydrophilic low-molecular molecules. An evidence for the existence of a glycoprotein WGA receptor on the surface of *A. lipoferum* SpBr17 has been obtained [9]. A WGA-binding glycoprotein with the molecular mass of 32 kDa has been identified in the extracellular material of strain SpBr17; its elimination through mutagenesis resulted in the loss of ability of the bacterium to bind WGA and respond to this stimulus by intensification of nitrogen fixation. Another example of a signal protein receptor localized on the bacterial surface, is the surface protein of mycobacteria binding the epidermal growth factor; it has been identified in mycobacteria and cloned in *E. coli* [2]. In *A. brasilense* Sp7, the most probable candidate for the role of WGA receptor is the surface glycoprotein of this strain, which is a lectin like WGA. The lectin of strain Sp7, which has been isolated from the bacterial surface and purified, binds WGA in the dot-blot analysis. This binding is inhibited not by haptens of the bacterial lectin (L-fucose and D-galactose) but by the WGA hapten: *N*-acetyl-D-glucosamine [18]. This data is in good agreement with our results showing that WGA preincubated with *N*-acetyl-D-glucosamine produces no changes in the EO parameters of azospirilla cell suspension, which may be interpreted as the absence of lectin binding with azospirilla cells (Fig. 3).

It is not improbable that WGA reception also involves capsular polymers: a lipopolysaccharide–protein complex, a polysaccharide–lipid complex, and a free polysaccharide (not included in the complexes). Each of the above three polymers has shown the ability for specific and reversible WGA binding [19].

Thus, we have demonstrated the possibility of using electrooptical analysis of bacterial suspensions for the study of the high-specific binding of polypeptide molecular signals with bacterial cells and for the estimation of the dynamics of this process. It should be noted that the proposed approach makes it possible to study the dynamics of the binding of other polypeptide signals with target cells. In particular, registration of the EO parameters of cell suspensions could give significant information about the binding of other interleukins with the cells of virulent strains of *E. coli* and *Salmonella typhimurium*; this approach may also be used for the study of the initial stages of interaction of the Rpf protein with *Micrococcus luteus, Mycobacterium tuberculosis*, or other bacteria competent to this signal.

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