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EXPERIMENTAL ARTICLES

Role of Sodium Ions and Their Uptake by Cells of Cultured Blue-Green Algae, *Spirulina platensis* and *Spirulina maxima*

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Abstract—The growth of the blue-green algae *Spirulina platensis* and *Spirulina maxima*, cultured in complete mineral Zarouk medium containing Na^+ or Na^+-deficient medium, was studied over a period of 24 h. The optical densities of *S. platensis* and *S. maxima* cells, determined during the last hour of exposure to sodium deficiency, amounted to 55.6 and 32.6%, respectively, of the optical densities of the same cells grown in complete Zarouk medium. Moreover, the cultures grown in Na^+-deficient medium exhibited increased ability to take up sodium (which was low in *S. platensis* and *S. maxima* cells cultured in complete mineral medium). It is concluded that the two species studied are characterized by periodic, on the order of minutes, changes in the cellular uptake and release of sodium.

Key words: *Spirulina platensis* and *Spirulina maxima*, Zarouk medium with altered sodium content, optical density of cells, Na^+ transport.

Autotrophic microorganisms inhabiting alkaline media need sodium. It is believed that sodium may be involved in processes of carbon and nitrogen assimilation, which ensure viability of microbial cells under these conditions [1].

One of the pathways of direct involvement of sodium in cellular energy transformations is related to processes in which sodium cations replace protons and act as primary coupling ions in the generation of the transmembrane potential difference [2].

The unique blue-green algae *Spirulina platensis* and *Spirulina maxima* live in alkaline media at pH 11 or higher [3]. It was of interest to study the growth of the two microorganisms in culture and their ability to transport sodium. In our previous studies of these cultures, exposure to sodium deficiency affected the cell growth and the concentrations of ions in the mineral medium no earlier than after 24 h. In this work, therefore, we sought to identify the earliest changes in the above parameters, observed in cultures of the alkaliphilic *Spirulina* species under the conditions of the absence of sodium in the medium. Furthermore, here we report on an alternative method used for preparing the cells.

MATERIALS AND METHODS

Used in this work were 15-day cultures of the blue-green algae *Spirulina* (*Arthrospira*) *platensis* (Nordstedt) Geitler (obtained form the collection of cultures of the Institute of Plant Physiology, Russian Academy of Sciences) and *Spirulina* (*Arthrospira*) *maxima* (Setchell a. Gardiner) Geitler. These morphologically similar algae serve as producers of valuable proteins (including forage protein) and biologically active substances [4].

The cultures were grown in 100-ml flasks, each containing 50 ml of Zarouk mineral medium. Conditions of *Spirulina* culturing were as described in our prior report [5].

To study the growth of the cultured cells and the transport of sodium ions (Na^+), two media were used, one containing the complete set of components and the other, Na^+-deficient (sodium salts were replaced by equimolar amounts of potassium compounds).

The layout of cell preparation for studies of growth and transmembrane Na^+ fluxes in the cells of 15-day cultures of the blue-green algae *S. platensis* and *S. maxima* is shown in Fig. 1. Aliquots of 25 ml of cell suspension were taken from each flask containing cultures of the algae grown in complete mineral medium. The cell were pelleted by centrifugation at 9000 *g* for 30 min, and the supernatant was discarded. The cells were resuspended in distilled water and pelleted once again under the same conditions. The supernatant was discarded, and the cells were used as material for inoculation into complete and Na^+-deficient medium. The inoculation was performed in such a way as to ensure that the optical density of the cell suspension in each flask be approximately the same (0.1–0.2).

The blue-green algae *S. platensis* and *S. maxima* were grown in complete and Na^+-deficient mineral

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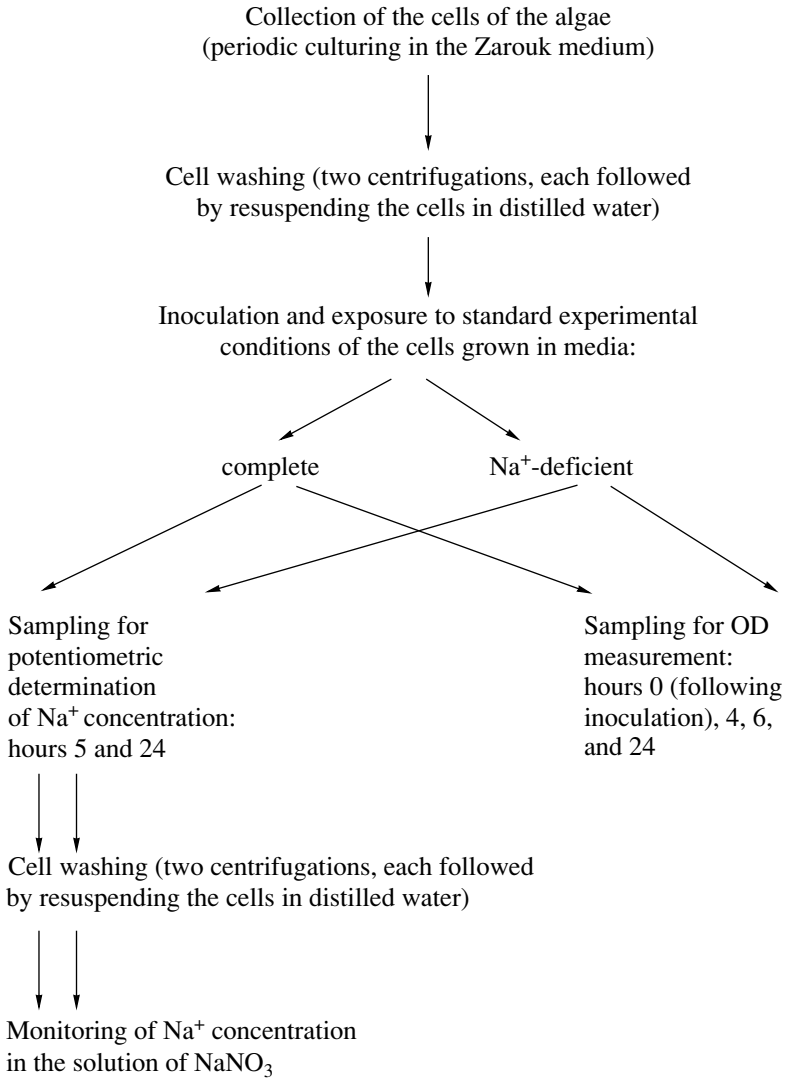

Fig. 1. Schematic representation of the preparation of *Spirulina* cells for studies of growth and sodium fluxes.

media for 24 h, under standard conditions. The growth of the suspension in each setting was characterized by changes in the OD at 540 nm. Measurements were performed within hours 2, 4, 6, and 24 of the culture growth using a KFK-2 photoelectric colorimeter equipped with 3.0-mm cuvettes.

To study Na^+ fluxes in the cells of the blue-green algae, samples of the cultures were withdrawn within hours 5 and 24 of the growth, centrifuged twice, and resuspended in distilled water. Uptake or release of sodium was characterized by changes in its concentration in a solution of the salt, $NaNO_3$ [6]. The setups for recording these changes were an Orion EA-920 ion meter (United States) and an ion-selective electrode, ESL-51-07 (Russia). The rate of uptake or release was measured as follows: 7 ml 5×10^{-2} M $NaNO_3$ was introduced into the chamber of the instrument, and the readout of the ion meter corresponding to this concentration of Na^+ was recorded; thereafter, 2 ml of the cell suspension in distilled water were added into the chamber, and the new readout (corresponding to the dilution 7 : 9) was recorded, followed by monitoring its changes in light (for 10 min) and in the dark (for 10 min). The uptake or release of sodium, calculated from the difference between the original and final concentrations, was expressed in mol l^{-1} per 10 min (related to 2 ml of a cell suspension having an OD of 1. The experiments were run in duplicate, triplicate, or quadruplicate. The figures show data of representative experiments; the results have been statistically tabulated.

RESULTS AND DISCUSSION

According to the data reported in [7], a component in the absence of which cessation of growth of a blue-green alga occurs, is defined as an essential element of

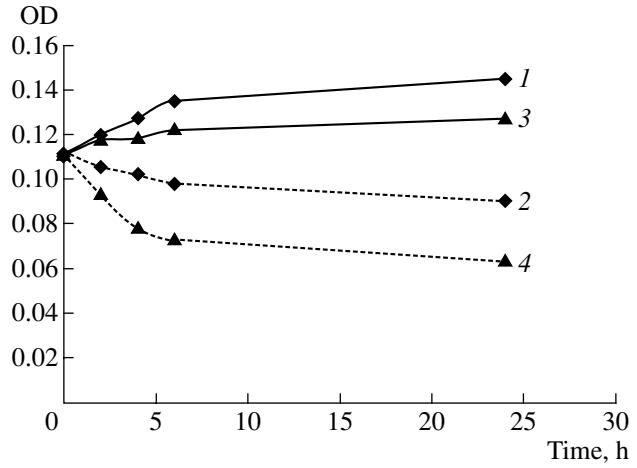

Fig. 2. Kinetics of optical density of suspensions of *S. platensis* and *S. maxima*, grown in complete or Na^+-deficient medium for 24 h: *1*, *S. platensis* in complete medium; *2*, *S. platensis* in Na^+-deficient medium; *3*, *S. maxima* in complete medium; *4*, *S. maxima* in Na^+-deficient medium.

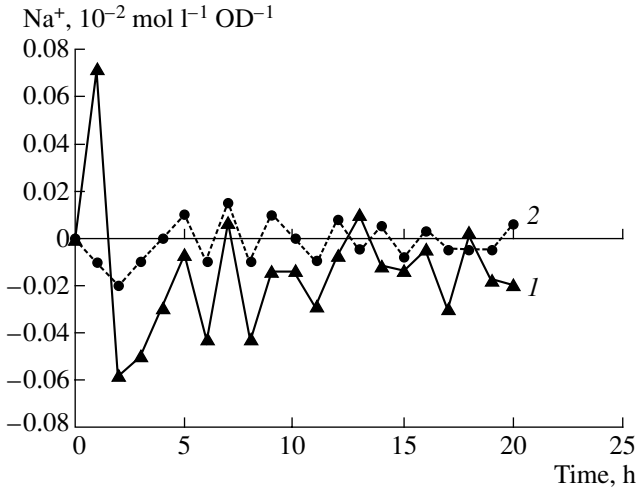

Fig. 3. Kinetics of content of sodium cations, released or taken up by the cells of *S. maxima*, grown in complete or Na^+-deficient media: *1*, *S. maxima* in complete medium; *2*, *S. maxima* in Na^+-deficient medium. Na^+ release corresponds to negative values of ordinates.

mineral nutrition for this organism. Sodium is a proven essential element of mineral nutrition for *Anabaena variabilis*, which grows under neutral conditions [8], and sodium is known to stimulate its growth. In the case of the alkaliphilic *Spirulina* spp., the need for sodium has not been addressed in sufficient detail.

Figure 2 shows the kinetics of OD of cultured *S. platensis* and *S. maxima* cells grown in the presence or in the absence of sodium in the medium. It is clear that the values of OD of the cultures subjected to different growth conditions underwent different changes throughout the 24 h of the experiment. For example, the growth of *S. platensis* and *S. maxima* in the complete medium was associated with steady increases in OD values, the first culture being notably predominant in this respect. Conversely, under the conditions of Na^+ deficiency, both *S. platensis* and *S. maxima* exhibited decreases in OD values, which became obvious in less than 2 h after the onset of the experiment (even though the appearance of blue-green algae grown in complete and Na^+-deficient media was exactly the same).

During hour 6 of exposure to Na^+ deficiency, both *Spirulina* cultures changed the characteristic dark-green color into yellowish-green, which was likely associated with the development of chlorosis. Of note, chlorosis was more pronounced in *S. maxima* than *S. platensis*. Similar results (appearance of chlorosis under the conditions of Na^+ deficiency) were reported previously in experiments with *A. variabilis* [8]. In the latter case, chlorosis, attended by cell death and lysis, took place only in alkaline medium (at pH 8.2 or higher). (Alkalinity was not in itself deleterious to *Anabaena*, because no indices of chlorosis could be observed at the same pH values if sodium was present in the medium.)

By the end of the experiment (during hour 24), the values of OD in *S. platensis* and *S. maxima* cells exposed to Na^+ deficiency amounted to 55.6 and 32.6%, respectively, of those recorded for their counterparts grown in complete medium. Because the decrease in OD values and the development of chlorosis are underlain by the lack of sodium in the mineral medium, it is reasonable to suggest that this cation is an essential component of mineral nutrition for blue-green algae, critical for their normal growth. Data from the literature indicate that the need for this element is specific, in that its replacement by other monovalent cations does not replenish the deficiency. Supporting evidence is provided in a report describing the effects of cations (Na^+, K^+, Cs^+, and Mg^{2+}) on photosynthesis [9].

It is well known that *Spirulina* spp. represent alkaliphilic microorganisms and their need for sodium manifests itself at high pH values. The concentration of this element in mineral Zarouk medium, optimum for these blue-green algae, is 0.209 mol l^{-1} (this is 11.6 times higher than the content of the same cation in mineral BG11 medium, optimum for *A. variabilis*). Moreover, blue-green algae of the genus *Spirulina* are classified with the so-called sodium microorganisms [10], based on their utilization of the sodium cycle in the membrane.

Figure 3 shows that the content of Na^+ in the solution of $NaNO_3$ underwent oscillations throughout the whole period under study (20 min), which were underlain by inward and outward cellular fluxes. This phenomenon is a presentation of periodic, on the order of minutes, changes in the cellular uptake and release of sodium, which are characteristic of *Spirulina* spp. The amplitude of these oscillations was higher in light than in the dark, indicating that processes of Na^+ uptake and

Rates of uptake and release of sodium ions by the cells of *S. platensis* and *S. maxima* exposed to light

	S. platensis	*S. maxima*
Variant of the experiment	Uptake/Release, mol l^{-1} per 10 min	Uptake/Release, mol l^{-1} per 10 min
Complete medium		
5-h exposure	0.138 ± 0.02/0.076 ± 0.06	0.064 ± 0.04/0.187 ± 0.07
24-h exposure	0.049 ± 0.04/0.103 ± 0.08	0.067 ± 0.02/0.160 ± 0.02
Na^+-deficient medium		
5-h exposure	0.129 ± 0.06/0.032 ± 0.02	0.055 ± 0.02/0.040 ± 0.03
24-h exposure	0.273 ± 0.07/0.203 ± 0.02	0.101 ± 0.06/0.049 ± 0.05

release by cells are light-dependent, which lends further support to our previously reported findings [11].

The tabulated data indicate that exposure to light of *S. platensis* and *S. maxima*, grown in Na^+-deficient media, stimulates Na^+ uptake. It is conceivable that the cultures lacking sodium were actively taking up this mineral component, thereby satisfying their need. Conversely, growth of the blue-green algae in complete medium was, in most cases, associated with a weak capacity for Na^+ uptake. This is likely the consequence of the high intracellular content of the cation.

Thus, cultures of *Spirulina* spp. growing under alkaline conditions require sodium for normal growth. The amount of Na^+ taken up the cells of the blue-green algae is determined by the culturing conditions, which in turn affect the physiological status of the culture. *Spirulina* spp. growing in Na^+-deficient medium (and therefore lacking sodium) actively took up this mineral component, thereby satisfying their physiological need.

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