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Light-dependent $\Delta\bar{\mu}Na$-generation and utilization in the marine cyanobacterium *Oscillatoria brevis*

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Light-dependent Na^+ and H^+ transports, membrane potential ($\Delta\Psi$) and motility have been studied in the cells of the marine cyanobacterium *Oscillatoria brevis*. In the presence of a protonophorous uncoupler, carbonyl cyanide-*m*-chlorophenylhydrazone, the intracellular Na^+ level is shown to increase in the dark and decrease in the light. The Na^+/H^+ antiporter, monensin, stimulates the dark CCCP-dependent $[Na^+]_{in}$ increase and abolishes the light-dependent $[Na^+]_{in}$ decrease. Na^+ ions are necessary for the fast light-induced $\Delta\Psi$ generation and H^+ uptake by the cells. This uptake is inhibited by monensin being resistant to CCCP. Monensin sensitizes the $\Delta\Psi$ level and the motility rate to low CCCP concentrations. The obtained data are consistent with the assumption that *O. brevis* possesses a primary Na^+ pump which utilizes (directly or indirectly) the light energy.

Na^+ energetics; Cyanobacteria; *Oscillatoria brevis*

1. INTRODUCTION

Bioenergetic studies carried out over the past 10 years have clearly shown that H^+ is not unique as a coupling ion. In some heterotrophic bacteria (aerobic or anaerobic), Na^+ ions have been found to effectively substitute for H^+ ions in all the types of membrane-linked energy transduction (reviewed in [1–6]). In our group, some indications were obtained that a marine phototrophic microorganism, the cyanobacterium *O. brevis*, seems to employ the Na^+ cycle in its plasma membrane when the light energy is used for generating $\Delta\Psi$ and energizing the motility mechanism [7]. In this paper we have studied Na^+ and H^+ transports, as well as $\Delta\Psi$ generation and motility, in the absence and in the presence of a protonophorous uncoupler and monensin. The obtained data confirmed the suggestion that there is a primary Na^+ pump in the *O. brevis* plasma membrane.

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Abbreviations: $\Delta\bar{\mu}H$ and $\Delta\bar{\mu}Na$, transmembrane electrochemical H^+ and Na^+ potential differences, respectively; $\Delta\Psi$, transmembrane electric potential difference; ΔpH and ΔpNa, transmembrane H^+ and Na^+ concentration differences, respectively; CCCP, carbonyl cyanide-*m*-chlorophenyl hydrazone; $[Na^+]_{in}$, intracellular Na^+ concentration; TPP^+, tetraphenylphosphonium

2. MATERIALS AND METHODS

O. brevis (Kutz) gom [8,9] was isolated by the authors from the salt-water Kuyalnic lagoon (near Odessa, USSR). The pure culture of *O. brevis* (strain 5, early BKG-1, the Odessa State University cyanobacterial collection) was grown under periodic illumination in the ASN III medium [9]. In the late exponential growth phase, cells were harvested. The growth rate of the cyanobacterium was determined by monitoring chlorophyll concentration measured as in [10].

Measurements of the motility rate and the $\Delta\Psi$ level of intact trichomes were carried out in the growth medium or in Medium 1, containing 10 mM $MgCl_2$, 10 mM $KHCO_3$, 5 mM KCl and 10 μM $MnCl_2$, pH 9.0–9.6. To measure the Na^+ and H^+ transports, trichomes were broken by shaking in the growth medium and fragments of trichomes (50–100 cells each) were harvested by centrifugation, washed and resuspended in the experimental medium. The Na^+ transport was measured in Medium 2, containing 400 mM NaCl, 10 mM KCl, 5 mM $MgSO_4 \times 7H_2O$, 10 mM Tris, pH 9.2. For pH measurements, we used the same medium but without Tris (medium 3). Studying the effects of different NaCl concentrations, we added mannitol to keep the tonicity level constant. For illumination, the light of a 75 W halogen lamp was used.

The $\Delta\Psi$ level across the cytoplasmic membrane in a single *O. brevis* cell was determined by means of the fluorescent penetrating cation ethylrodamine and microfluorimeter [7,11,12]. In a microscopy study, trichomes were put onto the cover glass. After 12 h, most of the trichomes were found to be attached to the glass. When indicated, the trichomes were preincubated in the presence of the ionophore for 1 h. The rate of trichome motility was measured as described in [7].

To measure intracellular Na^+, 1 ml suspension of trichomes (~16 μg chlorophyll $\times$ ml^{-1}) was loaded onto a nitrocellulose Synpor filter (Czechoslovakia; the diameter of the pores was 2.5 nm) and washed twice with 0.5 M mannitol. All the manipulations took no more than 5 s. The Na^+ contents were measured by means of a 'Flavo' flame photometer. The pH level in the cell suspension containing approx. 4 μg chlorophyll $\times$ ml^{-1} was monitored with a radiometer pH electrode in Medium 3.

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$[Na^+]_{in}$ was evaluated assuming that the internal water space was 140 ± 20 μl × mg chlorophyll^{-1}, which was estimated as in [13], using tritium water (0.2 mCi/g) and hydroxy[^{14}C]methyl inulin (13.8 mCi/mmol).

3. RESULTS

Earlier we showed that the maximal growth of *O. brevis* took place at 200–400 mM NaCl and pH 9.0–9.6 [12]. Under these conditions we performed all the experiments described below.

As is shown in Fig. 1, an [NaCl] increase in the incubation medium leads to an increase in the rate of $\Delta\Psi$ generation on the *O. brevis* plasma membrane, measured by ethylrodamine fluorescence. In this experiment, the medium without NaCl contained mannitol instead of NaCl so as to sustain the tonicity level. In another experiment, NaCl was replaced by KCl. Here $\Delta\Psi$ generation was also much slower than with NaCl. Even a more pronounced Na^+ effect on $\Delta\Psi$ was observed in the medium supplemented with the protonophorous uncoupler CCCP (not shown). The favorable Na^+ effect was decreased by adding the Na^+/H^+ antiporter monensin. This ionophore was found to increase $\Delta\Psi$ without CCCP and to decrease $\Delta\Psi$ in the presence of CCCP (Fig. 2). In the same figure, it is shown that monensin sensitizes *O. brevis* motility to the inhibitory action of low CCCP concentrations.

Fig. 3 demonstrates the results of Na^+ level measurement in *O. brevis* cells. It is seen (Fig. 3A) that without added ionophores, the cells maintain low $[Na^+]_{in}$ in the dark so that illumination decreases $[Na^+]$ only slightly. The addition of CCCP, especially together with monensin, induces a strong increase in the $[Na^+]_{in}$ level in darkness. Under illumination, $[Na^+]_{in}$ rapidly decreases in the sample with CCCP. This effect is arrested by monensin so that the $[Na^+]_{in}$ level remains high even in the light, if both CCCP and monensin are present.

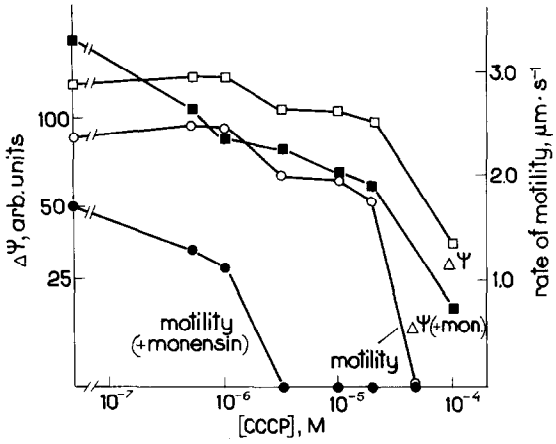

Fig. 2. Effects of CCCP and monensin on the $\Delta\Psi$ level and motility of *O. brevis*. Medium 1 containing 50 mM NaCl, pH 9.6. Addition, 3 μM monensin.

The monensin concentration used in this experiment (4 μM), when taken without CCCP, was without measurable effect upon $[Na^+]_{in}$. At the same time, 14 μM monensin induced a strong $[Na^+]_{in}$ increase in the dark. Subsequent illumination failed to decrease $[Na^+]_{in}$ (Fig. 3B).

Fig. 4 shows the pH responses of *O. brevis* cells. It is seen that the light-induced pH change is very small in the absence of added Na^+. In the presence of Na^+, illumination causes a significant H^+ uptake which is reversed in the dark. The addition of CCCP stimulates, rather than inhibits, this response which is strongly suppressed when the medium is supplemented with 3 μM

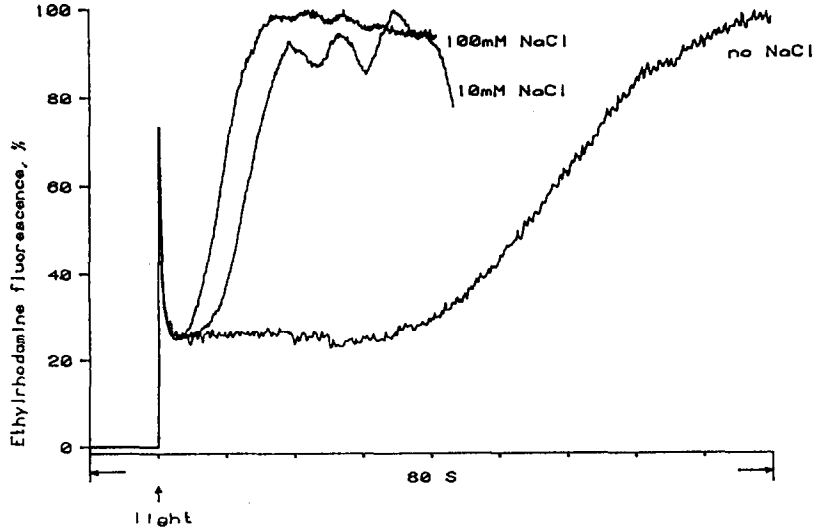

Fig. 1. Effect of different NaCl concentrations upon the $\Delta\Psi$ level in *O. brevis*. The fluorescent signal was measured in one of the trichome-composing cells. Medium 1, pH 9.0. The fluorescence increase attests on the electrophoretic ethylrodamine accumulation inside the cell.

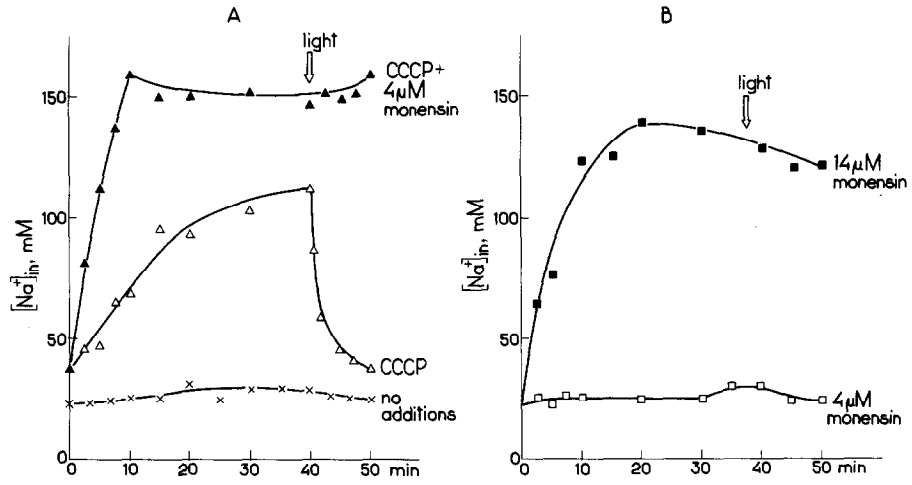

Fig. 3. Effects of CCCP, monensin and light upon the $[Na^+]_{in}$ level in *O. brevis* cells. Cyanobacteria (16 μg chlorophyll × ml^{-1}) was incubated in medium 2. Additions, 20 μM CCCP, 4 or 14 μM monensin.

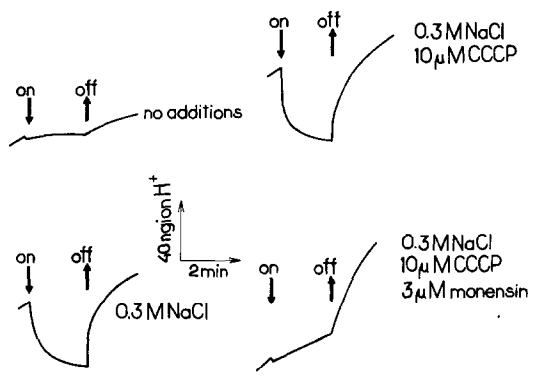

Fig. 4. Light-induced H^+ uptake by *O. brevis*. Medium 3, pH 9.2, containing 1 mM methylviologen.

monensin. Without CCCP, this monensin concentration has only a slight effect on the light-induced pH response, whereas 10 μM monensin abolishes the response (not shown).

Light-induced, Na^+-stimulated H^+ uptake by *O. brevis* cells was abolished by 0.5 mM TPP^+ which did not affect the electron transfer in the photosynthetic electron transport chain (not shown).

4. DISCUSSION

The above-mentioned results are summarized in Eqns 1–4. It is supposed that in the cytoplasmic membrane of the marine cyanobacterium *O. brevis*, there is a primary Na^+ pump energized, directly or indirectly, by the light (Eqn. 1). The work of the pump results in the generation of $\Delta\Psi$ due to electrogenic Na^+ extrusion (see Fig. 1 for Na^+ dependence of $\Delta\Psi$ formation).

$$Na^+_{in} \xrightarrow[\Delta\Psi]{\text{primary } Na^+ \text{ pump}} Na^+_{out} \quad (1)$$

$$Na^+_{out} \xrightarrow[\Delta\Psi]{Na^+ \text{ motor}} Na^+_{in} \quad (2)$$

$$H^+_{out} \xrightarrow[\Delta\Psi]{H^+ \text{ channel; CCCP}} H^+_{in} \quad (3)$$

$$Na^+_{out} + H^+_{in} \xrightarrow{\text{monensin}} Na^+_{in} + H^+_{out} \quad (4)$$

Downhill movement of Na^+_{out} back to the cytoplasm supports the performance of the work by the $\Delta\tilde{\mu}_{Na}$ consumers in the cytoplasmic membrane (e.g. the operation of Na^+ motors [3,14,15], Eqn. 2, Fig. 2). The Na^+ pump-produced $\Delta\Psi$ can also be utilized to import H^+_{out}, i.e. to acidize the cytoplasm, a function essential for life under alkaline conditions (Eqn. 3). This import seems to be carried out by an H^+ channel or uniporter inherent in the *O. brevis* plasma membrane, and may be stimulated by CCCP (Fig. 4). The Na^+/H^+ antiporter monensin dissipates both the Na^+ and H^+ gradients (Figs 3 and 4, respectively, and Eqn. 4).

A crucial premise for the above concept is the conclusion that $\Delta\tilde{\mu}_{Na}$ between the *O. brevis* cytoplasm and the outer medium is generated by a primary sodium pump, rather than by an endogenous Na^+/H^+-antiporter. Such a conclusion is supported, first of all, by the data presented in Fig. 3A, namely, that the light-dependent Na^+ efflux can be shown in the presence of high concentrations of CCCP (Fig. 3A) which stimulates, rather than suppresses, the light-dependent H^+ uptake (Fig. 4). The resistance of the Na^+ efflux to CCCP is not due to the inefficiency of CCCP under the conditions used since CCCP: (1) stimulates the Na^+ influx in the dark; and (2) sensitizes the Na^+ efflux to low concentration of monensin (Fig. 3).

The involvement of the electroneutral endogenous

Na^+/H^+ antiporter, utilizing the $\Delta\tilde{\mu}_H$ produced by an H^+ pump, seems improbable since inhibition is the only effect of monensin on the light-induced Na^+ extrusion (Fig. 3) and motility (Fig. 2).

It seems noteworthy that all the above experiments were performed at alkaline pH (> or = 9.0), i.e. under conditions when the pH gradient is favorable for the Na^+ influx rather than efflux.

It is not clear what kind of a primary sodium pump operates in the cytoplasmic membrane of *O. brevis*. At least 3 possibilities should be considered: (1) Na^+-motive photosynthetic redox chain directly utilizing the light energy (see [3] for discussion); (2) a Na^+-motive respiratory chain [5,6,16] using reducing equivalents produced by the usual (H^+-motive) photoredox chain in thylakoids; and (3) a Na^+-motive ATPase [2,3] hydrolyzing the thylakoid-produced ATP. This problem is now under study.

The mechanism of the endogenous H^+ uniport is yet another problem. The electrophoretic character of the light-dependent H^+ efflux in the presence of Na^+ was confirmed by the fact that the penetrating cation TPP^+ discharging $\Delta\Psi$ was shown to inhibit the influx. It may be mentioned in this context that the H^+ uniporter, termogenin, has already been described in one of the animal tissues, namely, in brown fat (see [3] for review).

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