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**Na⁺ and water fluxes across cell membranes of the freshwater cyanobacterium *Synechococcus* as reported by chlorophyll *a* fluorescence**

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**Introduction**

Cell of fresh water cyanobacterial genus *Synechococcus* (e.g., *S.* sp. PCC 7942, hereafter S7942) import NaCl passively and export Na⁺ actively, primarily via Na⁺/H⁺ antiport. During a salinity upshock episode, the cell volume first increases to a maximum within 2-3 min as a result of passive importation of Na⁺, Cl⁻, and H₂O (rise branch), and then it decays slowly (tens of minutes) to a lower steady value as a result of active exportation of Na⁺ ions (decay branch; Nitschmann and Packer 1992). NaCl-induced volume changes of cyanobacterial cells are reported quantitatively by changes in the intensity of PBS-sensitized Chla fluorescence (Stamatakis et al 1999). In this research we used NaCl-induced Chla fluorescence changes in order to learn more about the active extrusion of Na⁺ ions by S7942 cells and the effects of Na⁺ channel blockers on Na⁺ traffic through plasma membranes.

**Materials and methods**

S7942 was cultured at 31 °C in BG11, buffered with 20 mM Hepes NaOH, pH 7.5 (basal medium) using white light (100 µE m⁻² s⁻¹), and 5% v/v CO₂ in air. After 4 days, the cells were transferred to fresh basal medium (20 µg Chla ml⁻¹) to be used for assays. DCMU, 20 µM, was added to samples in order to close the reaction centers of photosystem II. Chl *a* fluorescence was measured with a PAM fluorometer (H. Walz, Effeltrich, Germany) as in Stamatakis et al (1999). Samples (600 µL) were incubated in darkness (4 min), then the periodic fluorescence excitation (1 µs; 120 nE m⁻² s⁻¹).

![Fig. 1. Time courses of Chla fluorescence as a result of salinity upshock (Δ[NaCl] = 0.4 M) in S7942 cell suspensions at pH 6.5 and at pH 9.0. A, the rise branch, B the rise and the decay branches of the NaCl-induced Chla fluorescence kinetics.](image-url)
1.6 kHz; 650 nm; \(\Delta \lambda = 25\) nm) was turned on and after 30 s 200 \(\mu\)L NaCl solution (1.6 M in basal medium) was injected to effect \(\Delta [\text{NaCl}] = 0.4\) M. Chla fluorescence was detected at \(\lambda > 690\) nm. Chla concentrations were determined as in Stamatakis et al. (1999).

Results and discussion

Figure 1 shows Chla fluorescence kinetics of S7942 cells induced by a 0.4 M NaCl upshock. Kinetics comprise a fast rise (Fig. 1A) and a slower decay (Fig. 1B). The rise denotes osmotic cell swelling, due to passive uptake of Na\(^+\), Cl\(^-\), and H\(_2\)O. This was opposed by active Na\(^+\) ion extrusion by the cells. At least two active processes have been proposed, Na\(^+\)/H\(^+\) exchange (Na\(^+\)/H\(^+\)-antiport, Blumwald et al 1983) and Na\(^+\) translocation by ATPases (Ritchie 1992). Na\(^+\) extrusion depresses cytoplasmic osmolality mainly because carboxylic salts dissociate more completely than carboxylic acids \((\text{i.e., } K_{\text{RCHOOH}} >> K_{\text{RCHOONa}})\). At pH 9 \(([\text{H}^+] = 1\) nM), only Na\(^+\) translocation (pumping) occurred, since Na\(^+\)/H\(^+\)-antiport was inoperative for lack of external protons. Accordingly, the NaCl-induced Chla fluorescence rise was more extensive at pH 9.0 than at pH 6.5 \(([\text{H}^+] = 316\) nm), where both active Na\(^+\) extrusion processes opposed the passive influx of Na\(^+\), Cl\(^-\), and H\(_2\)O. For the same reason, the fluorescence decay branch was less extensive at pH 9.0 (Fig. 1B).

Figure 2 presents a kinetic analysis of the rise branch of the NaCl-induced fluorescence kinetics. By plotting \(\log([F_{\text{MAX}}-F_t]/[F_{\text{MAX}}-F_2])\) the rise could be split into two consecutive first-order processes. Rate constants and time constants appear in the Fig. 2 legend. Results show nearly equal rise rates at pH 6.5 and at pH 9.0.

Figure 3 illustrates the effects of light starvation and of Na-vanadate on the NaCl-induced kinetics of Chla fluorescence in S7942 samples. Light starvation (16 h) deprived cells from a major source of ATP, without depleting them of it completely because of ongoing respiration and other catabolic processes. Na-vanadate inhibits the Na\(^+\)-translocating ATPases.

At pH 6.5, NaCl induced more extensive fluorescence rises and less extensive fluorescence decays in light-starved cells and in Na-vanadate treated cells than in control cells. This could be the result of diminished active translocation of Na\(^+\) and H\(^+\), and of undiminished Na\(^+\)/H\(^+\) exchanges. At pH 9.0, the Na\(^+\)/H\(^+\) exchange was inoperative, while primary Na\(^+\) translocation was running, as indicated by the more extensive fluorescence rise, and the absence of fluorescence decay in the presence of Na-vanadate. The most extensive Chla fluorescence rise was obtained at pH 9.0 with light-starved samples (Fig. 3B). It may suggest the involvement of additional processes for ATP-dependent extrusion of Na\(^+\) ions.
Figure 4 shows the effects of phenytoin and lidocaine (local anaesthetics) on the NaCl-induced kinetics of Chla fluorescence. These compounds block voltage-gated Na\(^+\) channels in nerve and muscle cells. In the presence of 0.4 M NaCl, S7942 cells are depolarized, so if some Na\(^+\) traffic occurred through voltage-gated channels then anaesthetics could interfere with it.

According to results, the two compounds had no effect on the NaCl-induced rise of Chla fluorescence (downhill importation of Na\(^+\)) while they diminished the Chla fluorescence decay (uphill exportation of Na\(^+\)) slightly. Both compounds are secondary amines so they may act as protonophores to reduces the transmembrane \(\Delta p\text{H}\). Complete obliteration of the decay branch was observed in the presence of strong protonophores. Recently, Allakhverdiev et al. (200) reported that Na\(^+\) channel blockers partially prevented the inactivation of photosynthetic oxygen evolution upon long-time exposure of S7942 cells to elevated salinity (0.5 M NaCl).

Fig. 4. Effects of the Na\(^+\) channel blockers phenytoin (100 \(\mu\text{M}\)) and lidocaine (100 \(\mu\text{M}\)) on the NaCl-induced kinetics of S7942 cell suspensions.
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References


