S26-007

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

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Keywords: chlorophyll fluorescence, cyanobacteria, *Synechococcus*, Na^+ transport, water transport

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 H_2O (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 ^oC 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 CO_2 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};

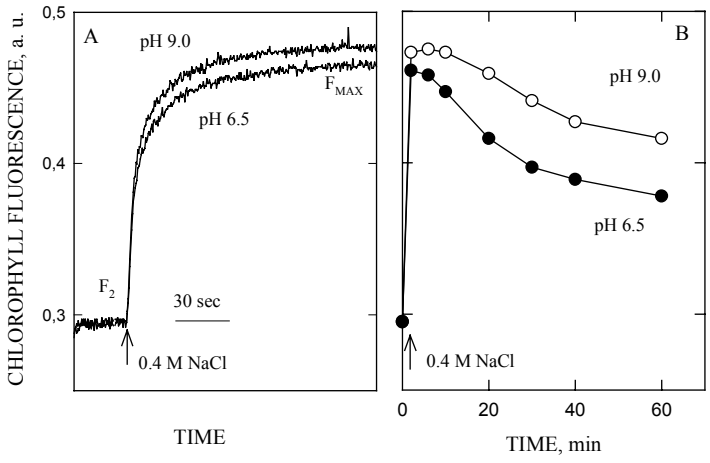

Fig. 1. Time courses of Chla fluorescence as a result of salinity upshock (Δ[NaCl] = 0.4 M) in S7942 cell suspensions at pH 6.5 and at pH 9.0. A, the rise branch, B the rise and the decay branches of the NaCl-induced Chla fluorescence kinetics.

1.6 kHz; 650 nm; $\Delta\lambda$ = 25 nm) was turned on and after 30 s 200 µL NaCl solution (1.6 M in basal medium) was injected to effect Δ[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_2O. 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 (*i.e.,* $K_{RCOONa} >> K_{RCOOH}$). At pH 9 ($[H^+]$ = 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 ($[H^+]$ = 316 nm), where both active Na^+ extrusion processes opposed the passive influx of Na^+, Cl^-, and H_2O. 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 $\log[(F_{MAX}-F_t)\ [(F_{MAX}-F_2)^{-1}]$ 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

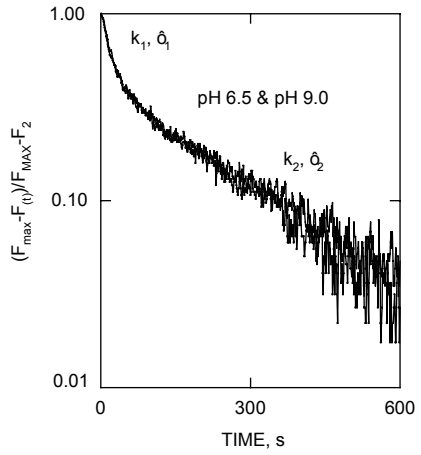

Fig. 2. Kinetic analysis of the NaCl-induced rise of Chla fluorescence in S7942 cell suspensions. Values of rate constants (k) and of half-time constants of the two consecutive first order processes are: At pH 6.5, $k_1 = 0.117\ s^{-1}$, τ_1 = 5.9 s and $k_2 = 0.021\ s^{-1}$, τ_2 = 33 s. At pH 9.0, $k_1 = 0.125\ s^{-1}$, τ_1 = 5.5 s and $k_2 = 0.024\ s^{-1}$, τ_2 = 28.9 s.

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.

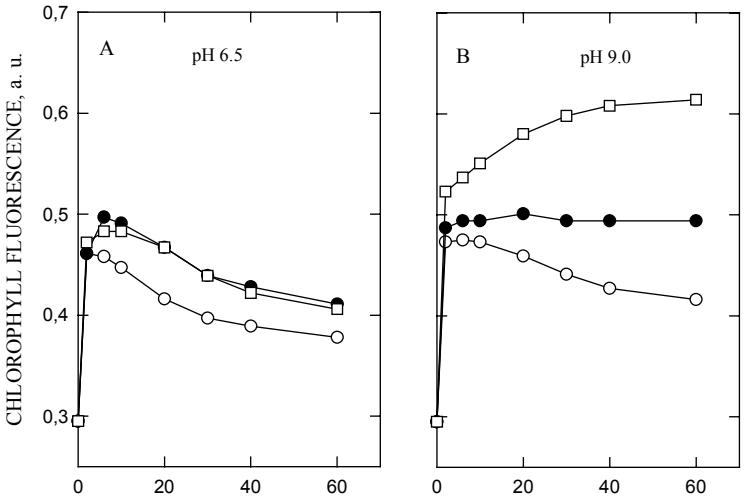

Fig. 3. Rise and decay branches of the NaCl-induced Chla fluorescence kinetics in S7942 cell suspensions. A: pH 6.5. B: pH 9.0. Control cells, no additions (○); presence of 1 μM Na-vanadate (●); light-deprived cells (16 h before harvesting, □).

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 ΔpH. Complete obliteration of the decay branch was observed in the presence of strong prtonophores. 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).

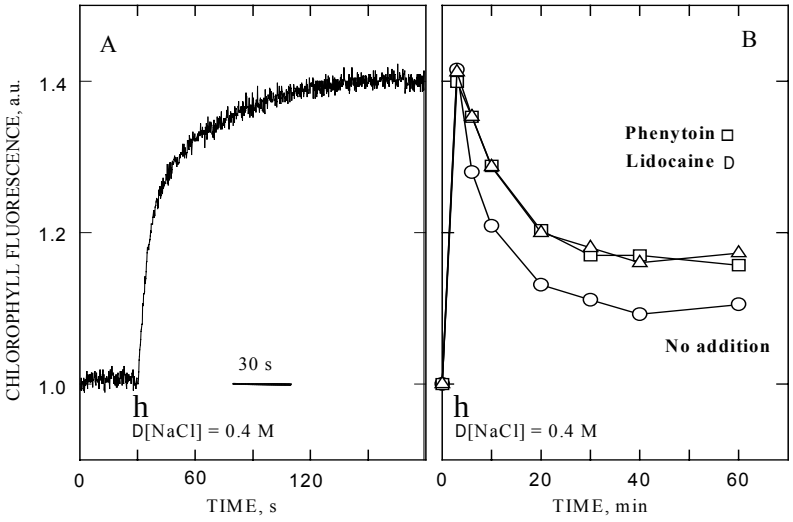

Fig. 4. Effects of the Na^+ channel blockers phenytoin (100 μM) and lidocaine (100 μM) on the NaCl-induced kinetics of S7942 cell suspensions.

Acknowledgements

Support by the Greek Ministry of Industry, Energy and Technology, and the European Social Fund (grant 99 EΔ 227) is gratefully acknowledged.

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