TEMPERATURE AND LIPID UNSATURATION EFFECTS ON PLASMA AND THYLAKOID MEMBRANES OF *SYNECHOCYSTIS* SP PCC6803

[1,2]Papageorgiou GC, [2,3]Govindjee, [2,3]Govindjee R, [2,4]Mimuro M, [1,2]Stamatakis K, [1]Alygizaki-Zorba A, [2]Murata N
[1]NRC Demokritos, Athens, Greece; [2]National Institute of Basic Biology, Okazaki, Japan; [3]University of Illinois Urbana-Champaign, IL, USA, [4]Yamaguchi University, Yoshida, Japan.

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1. Introduction

Membrane lipids of wild-type (WT) cells of cyanobacterium *Synechocystis* sp PCC6803 are characterized by an abundance of polyunsaturated fatty acids, but *desA*$^-$/*desD*$^-$ mutant cells contain saturated and monounsaturated fatty acids only (1,2). The difference in fatty acid unsaturation impacts on membrane fluidity. We probed here plasma membranes (PM) by inducing chlorophyll *a* (Chl *a*) fluorescence changes with osmolality shifts (3,4) and by measuring the electric conductivity in cell suspensions (5); and we probed the thylakoid membranes (TM) by inducing light/dark acclimative changes (state 1/2 transitions) of Chl *a* fluorescence (3,4).

2. Procedure

Synechocystis was cultured as in (2). The culture medium (BG11 plus 0.02 M Hepes NaOH, pH 7.5) was used also for assays and for sorbitol solutions of defined osmolality. Osmolalities were measured cryoscopically (3,4). Cell suspensions and suspension media were considered as equi-osmolal and are designated as hypo-osmotic or hyper-osmotic relative to the cell turgor threshold (maximal suspension osmolality at which cells retain turgor; 0.30-0.32 Osm kg^{-1} for *Synechocystis;* ref. 3).

Chl *a* fluorescence of samples (10-12 μg Chl ml^{-1} and 20 μM DCMU) preacclimated to darkness (4 min) was excited with weak modulated light (650 nm; $\Delta\lambda$ = 25 nm; 1.6 kHz; 1μs pulses; 70 nmoles m^{-2} s^{-1}). Cells were light-acclimated (transition to state 1) by actinic illumination (λ > 520 nm; 270 nmoles m^{-2} s^{-1}). Electric conductivity of cell suspensions during cooling/warming cycles (0.5 °C min^{-1}) was monitored as in (5). Fluorescence spectra at 77 K were recorded with a Hitachi 850 spectrofluorometer with 436

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nm light (to excite Chl *a* directly) or 580 nm light (to excite C-phycocyanin directly and Chl *a* indirectly). The excitation and emission bandwidths were 10 nm and 1 nm.

3. Result and Discussion

Figure 1A illustrates the experimental protocol and defines fluorescence magnitudes to be used. The modulated excitation generates fluorescence F_2. Light acclimation of cells raises fluorescence to F_1. Injection of suspension medium (arrow) depresses fluorescence to F_1' (iso-osmotic dilution 1:4). Removal of actinic light allows cells to reacclimate to darkness and fluorescence to drop to level F_2'. Fig. 1B repeats the protocol, except that samples were diluted (arrow) with sorbitol-containing medium to raise suspension osmolality by 0.905 Osm kg^{-1}. This depressed fluorescence more than the iso-osmotic dilution (Fig. 1A). $\Delta F'/F_2'$ relates to the osmotic contraction of cell volume (4), and it reports on PM functionality. In contrast, the light-acclimative fluorescence and $\Delta F/F_2$ report on TM functionality. With cells equilibrated at 34 °C, the most conspicuous difference in Chl *a* fluorescence occurred after a hyper-osmotic jump (Fig. 1B). WT cells experienced a transient hyper-osmotic quenching overshoot and then fluorescence rose to a steady level. In mutant cells, Chl *a* fluorescence was quenched by the hyper-osmotic jump to a steady lower level. The quenching overshoot must reflect a higher flexibility of PM in WT cells, which are richer in polyunsaturated fatty acids and more fluid than the PM of mutant cells.

Figure 2 shows a similar experiment performed with cells adjusted to 10 °C. $\Delta F/F_2$ and $\Delta F'/F_2'$ were now less extensive than at 34 °C, particularly in mutant cells whose TM undergo reversible thermotropic transition at 20.5 °C (data not shown). No analogous transition was detected in the TM of WT cells and in the PM of both strains. Also, there

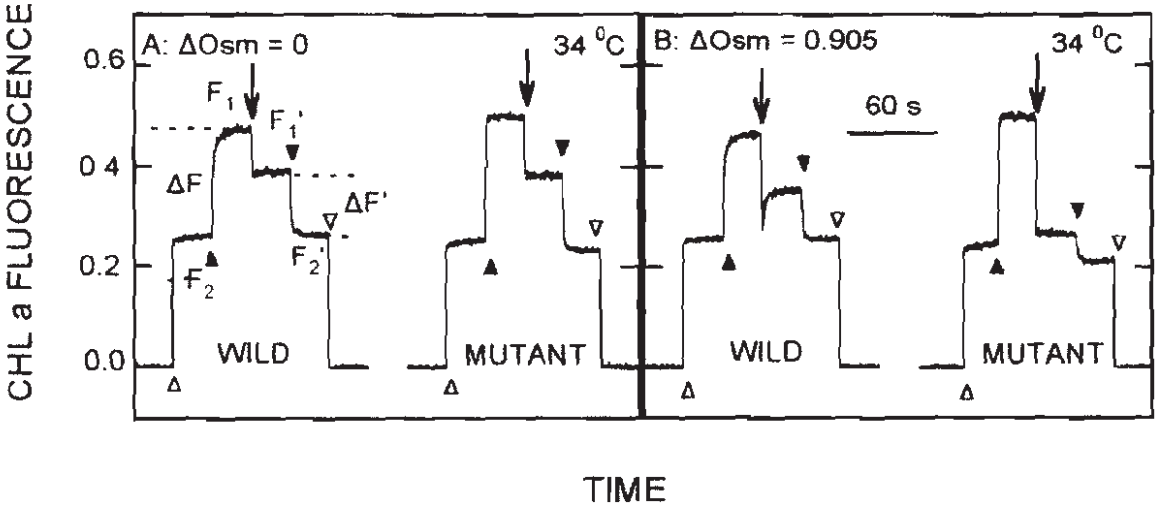

Fig. 1. Light-induced and osmotically-induced changes of Chl *a* fluorescence in WT and mutant *Synechocystis* cells at 34 °C. Δ,∇ indicate on, off times for the modulated light; ▲, ▼ indicate on, off times for the continuous actinic light. Arrows indicate sample dilution times. Symbols F_1, F_2, F_1', F_2', ΔF, and $\Delta F'$ are defined in panel A.

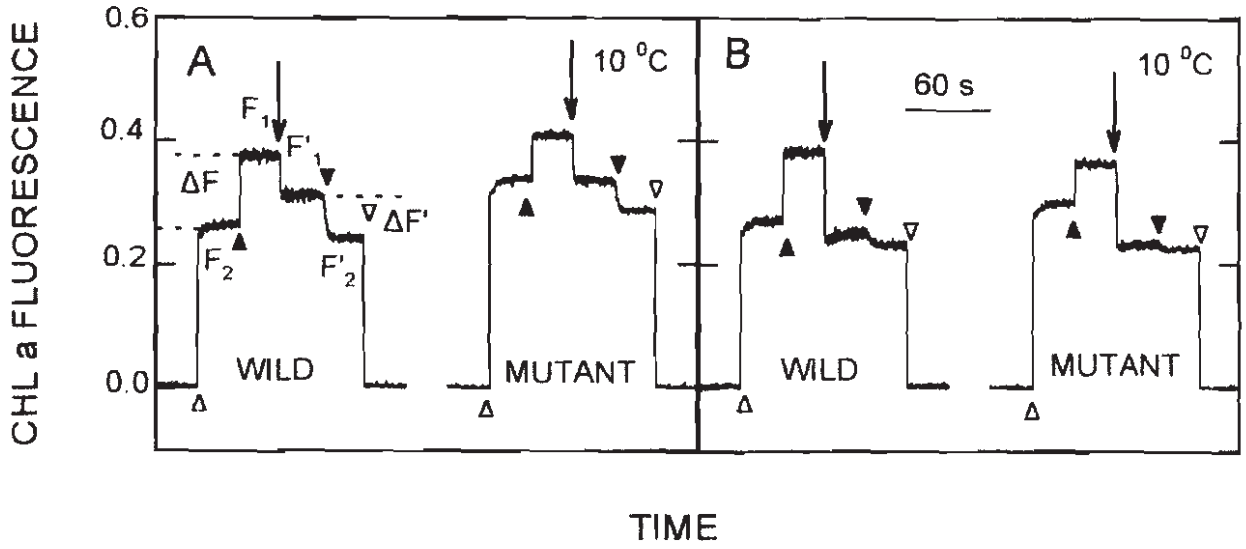

Fig. 2. Light-induced and osmotically-induced changes of Chl *a* fluorescence recorded with WT and mutant *Synechocystis* cells at 10 ° C. Details as in Fig. 1.

was no hyper-osmotic fluorescence quenching overshoot (Fig. 2B).

In the Fig. 3 we tested WT and mutant cells for irreversible chilling damage in two independent ways. In Fig. 3A we examined the chilling effect on $\Delta F/F_2$ (TM reporter) and on $\Delta F'/F_2'$ (PM reporter). Cells were chilled to 10 °C for various time and then they were returned to 34 °C for the fluorescence assay. No permanent damage occurred for up to 2 h chilling. In Fig. 3B we monitored the electric conductivity of cell suspensions during chilling/rewarming cycles (26 °C → 2 °C → 26 °C). There were no discontinuities in the temperature curves of electric conductivity to evidence uncontrollable leakage of

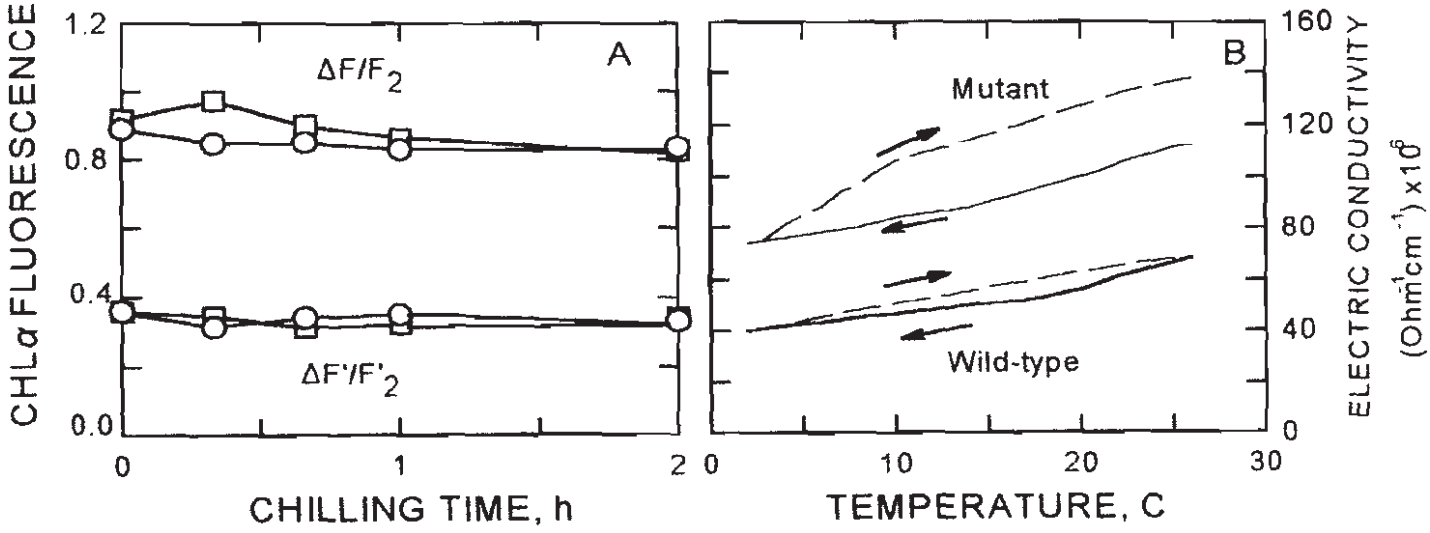

Fig. 3. Effects of chilling on Chl *a* fluorescence and electric conductivity of *Synechocystis* cell suspensions. (A) $\Delta F/F_2$ and $\Delta F'/F_2'$, measured at 34 °C, for WT(○) and mutant cells (□), as a function of chilling duration at 10 °C. (B) Electric conductivity of cell suspensions during chilling/rewarming cycles (26 °C to 2 °C).

cytoplasmic electrolytes.

Fig. 4 shows differences in the 77 K fluorescence spectra of hypo-osmotic and hyper-osmotic suspensions of WT cells. With 436 nm excitation (Fig. 4A) the spectrum of the hypo-osmotic suspension appears to be slightly red-shifted relative to the spectrum of the hyper-osmotic suspension. With 580 nm (B) in addition to the red shift, the hypo-osmotic suspension emitted excess fluorescence at 666 nm (phycobilisome), 685 nm and 696 nm (photosystem II). Thus hyper-osmotic media may partially impede electronic excitation transfers from phycobilisome to photosystem II.

Light-acclimative changes in cyanobacteria involve protein mass displacements within the TM phase (6). Osmotic cell volume changes involve expansion/shrinking of PM. Both these membrane processes are shown in this paper to be regulated by the degree of fatty acid unsaturation of membrane lipids.

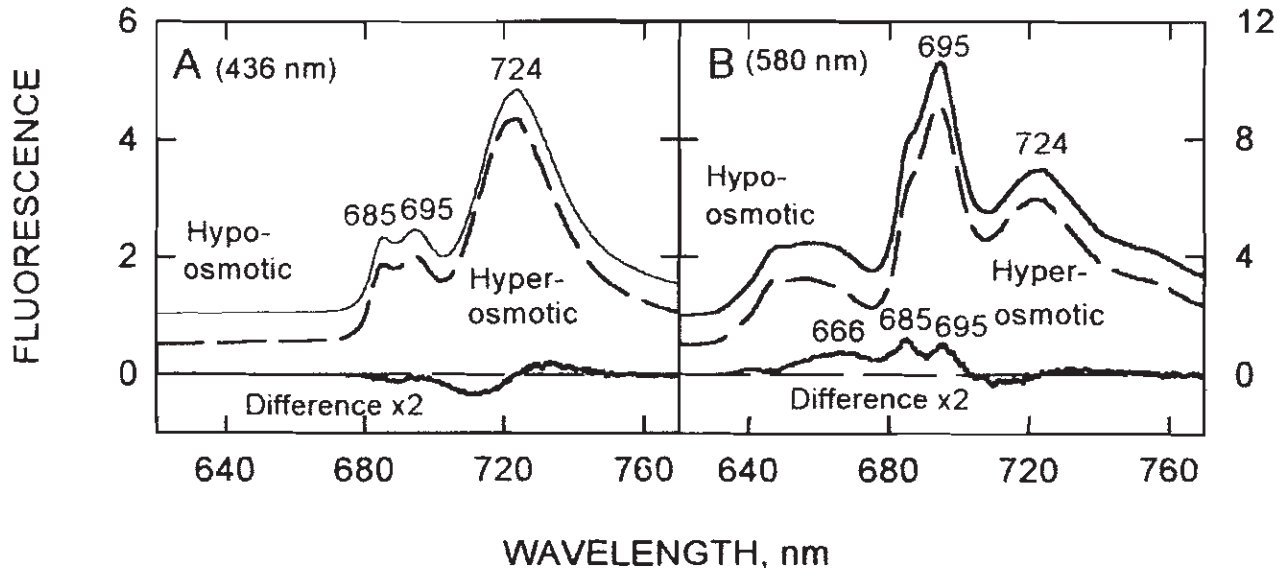

Fig. 4. Fluorescence spectra at 77 K of WT cells. Excitations 436 nm (A) or 580 nm (B). Light-acclimated cells in BG11 were frozen in liquid nitrogen either before (hypo-osmotic suspensions; solid lines) or after osmotic upshock (ΔOsm = 0.91 Osm kg^{-1}; hyper-osmotic suspensions; dashed lines). Spectra are normalized at 724 nm and shifted in the ordinate for clarity. Difference spectrum : hypo-osmotic minus hyper-osmotic.

References

1 Wada, H., Gombos, Z., Sakamoto, T. and Murata, N. (1992) Plant Cell Physiol. 33, 535-540

2 Tasaka, Y., Gombos, Z., Nishiyama, Y., Mohanty, P., Ohba, T., Ohki, K. and Murata, N (1996) EMBO J. 15, 6416-6425

3 Papageorgiou, G.C. and Alygizaki-Zorba, A. (1997) Biochim Biophys Acta 1335, 1-4

4 Papageorgiou, G.C., Alygizaki-Zorba, A., Ladas, N. and Murata, N. (1998) Physiol. Plant. (in press)

5 Murata, N., Wada, H. and Hirasawa, R. (1984) Plant Cell Physiol 25, 1027-1032

6 Bald, D., Kruip, J. and Roegner, M. (1996) Photosynth. Res. 49, 103-118