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Light-induced and osmotically-induced changes in chlorophyll *a* fluorescence in two *Synechocystis* sp. PCC 6803 strains that differ in membrane lipid unsaturation

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Abstract

Membranes of wild-type (WT) cells of the cyanobacterium *Synechocystis* sp. PCC 6803 are abundant in polyunsaturated fatty acids in membrane lipids and thus more fluid than membranes of *desA*$^-$*/desD*$^-$ mutant cells which contain no polyunsaturated fatty acids. Using intact cells we examined the effects of normal and chilling temperatures on membrane fluidity-dependent properties. We probed the thylakoid membranes by inducing light/dark acclimative changes in chlorophyll *a* (Chl *a*) fluorescence; and we probed the plasma membranes either by suppressing the Chl *a* fluorescence of light-acclimated cells under hyper-osmotic conditions, or by measuring the electric conductivity of cell suspensions. Thylakoid membranes of mutant cells undergo reversible thermotropic transition between 19 °C and 22 °C (midpoint at 20.5 °C). No analogous transition was detected in the thylakoid membranes of WT cells in the temperature range from 2 to 34 °C. Plasma membranes of both WT and mutant cells did not experience thermotropic transition in the temperature range from 2 °C to 34 °C as detected either fluorimetrically or by means of electric conductivity. Hyper-osmotic conditions caused fast transient fluorescence quenching in WT cells at 34 °C, but not at 14 °C, and not in mutant cells at either 34 °C or 14 °C. This transient quenching sensed probably the higher fluidity of the plasma membranes of WT cells. Hyper-osmotic media and dark acclimation had similar effects on the 77 K fluorescence of *Synechocystis* cells: they suppressed the ratio of photosystem II fluorescence to photosystem I fluorescence.

Abbreviations: Chl – chlorophyll; DCMU – 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Hepes – N-2-hydroxyethyl piperazine-N′-ethane sulfonic acid; PBS – phycobilisome; PS I, PS II – Photosystem I, Photosystem II; WT – wild-type

Introduction

In oxygenic photosynthetic organisms (plants, algae, cyanobacteria and prochlorophytes) the yield of chlorophyll (Chl) *a* fluorescence is subject to photochemical as well as to nonphotochemical quenching processes (see reviews by Govindjee 1995; Joshi and Mohanty 1995; Strasser et al. 1995; Papageorgiou 1996). Photochemical quenching relates directly to the turnover of PS II reaction centers. It ceases when photosynthetic electron transport is blocked by inhibitors (e.g., DCMU) that prevent spontaneous reoxidation of Q_A^-, the reduced form of the plastoquinone electron acceptor of Photosystem II (PS II), by intersystem intermediates. Nonphotochemical quenching is not related directly to the turnover of the PS II reaction

center. It is caused by various environmental stimuli that are classified as *short-term* and *long-term*, relative to the time it takes them to manifest. Among the long-term nonphotochemical modifiers of Chl *a* fluorescence are: zeaxanthin in the xanthophyll cycle of higher plants (Demmig-Adams and Adams 1992; Gilmore and Govindjee 1999); chromatic adaptation in cyanobacteria (Fujita et al. 1994); and complementary chromatic adaptation in cyanobacteria (Grossman et al. 1994).

A short-term nonphotochemical modifier of Chl *a* fluorescence *in vivo* is the acclimation of photosynthetic organisms to light or darkness. After a period of darkness (Papageorgiou and Govindjee 1967; Govindjee and Papageorgiou 1971) or after acclimation to light that activates PS II (Fork et al. 1979) cyanobacterial cells emit weaker Chl *a* fluorescence than after acclimation to light that activates PS I (Fork et al. 1979). The state of weaker fluorescence is known as *light state 2* and the state of stronger fluorescence *light state 1* (Murata 1969; Bonaventura and Myers 1969). In light state 2 more electronic excitation is diverted to the weakly fluorescent Chl *a* of PS I than in light state 1; in light state 1 more electronic excitation is diverted to the strongly fluorescent Chl *a* of PS II than in light state 2 (Fork and Satoh 1986; Williams and Allen 1987). The redistribution of the excitation energy during light/dark acclimation can be detected by the fluorescence spectra at 77 K (Murata 1969; Fork and Satoh 1986; Delphin et al 1996) or by the fluorescence decay-associated spectra of cyanobacterial cells at room temperature (Mullineaux and Holzwarth 1991).

Recently, another short-term nonphotochemical modifier of Chl *a* fluorescence of cyanobacteria has been identified (Papageorgiou and Alygizaki-Zorba 1997; Papageorgiou et al. 1998): the suppression of Chl *a* fluorescence of light-acclimated cyanobacterial cells when they are exposed to hyper-osmotic pressure with impermeable solutes. In contrast the Chl *a* fluorescence of dark-acclimated cells was insensitive to the hyper-osmotic environment.

Biological membranes are functional as long as they are fluid (see Quinn 1988; Murata and Wada 1995; Murata and Nishida 1996; Murata and Los 1997). When cooled below the temperature at which glycerolipid bilayers pass from the liquid-crystalline state to the phase-separated state, membranes become nonselectively permeable and lose biological function. Phase transition temperatures are lower when the membranes are richer in polyunsaturated fatty acids. When cyanobacterial cells are cultured in the upper range of tolerated temperatures, saturated fatty acids become abundant, and correspondingly the phase transition temperature shifts upwards. Conversely, when cyanobacterial cells are cultured in the lower range of tolerated temperatures unsaturated fatty acids become abundant and the phase transition temperature shifts downwards (Murata 1999).

Movement of integral proteins in the thylakoid membrane has been implicated in the light/dark acclimative transitions of cyanobacteria (Mullineaux 1994; Bald et al. 1996). Intuitively, we would expect such events to be influenced by membrane fluidity but convincing experimental proof was provided by Fork et al. (1979) and Fork and Satoh (1983). The possibility of alternate phycobilisme (PBS) docking and integral photosystem rearrangements would predict different absorption cross sections for the pigment antennae of PS II and PS I in light states 1 and 2. This has been also confirmed experimentally (Fork and Satoh 1986; Mullineaux and Holzwarth 1991).

By inactivating specific acyl-lipid desaturases in the cyanobacterium *Synechocystis* sp. PCC 6803, Wada and Murata (1989) and Tasaka et al. (1996) succeeded in selectively rectifying the $\Delta 6$, $\Delta 12$ and $\Delta 15$ ($\omega 3$) double bonds of the fatty-acyl moieties of glycerolipids. The most pronounced differences were detected in the *desA*$^-$/*desD*$^-$ mutant cells whose membrane glycerolipids lack polyunsaturated fatty acids and contain only $\Delta 9$-unsaturated and saturated fatty acids. In contrast, membrane glycerolipids of WT cells contain 66% of di-, tri- and tetra-unsaturated fatty acids with double bonds at the $\Delta 15$, $\Delta 12$, $\Delta 9$ and $\Delta 6$ positions. The dramatic difference in fatty-acid saturation between WT and *desA*$^-$/*desD*$^-$ mutant cells, and hence in membrane fluidity, is reflected in the phase transition temperature of thylakoid membrane lipids (Tasaka et al. 1996), the slower proliferation of mutant cells below 25 °C (Wada et al. 1992; Tasaka et al. 1996), and the defective recovery of the PS II function after photoinhibitory damage (Gombos et al. 1994; Kanervo et al. 1997).

In the present research, we compared WT and *desA*$^-$/*desD*$^-$ mutant cells of *Synechocystis* with respect to: (a) the light acclimative rise of Chl *a* fluorescence (light state 2 – light state 1 transition) and the dark acclimative decay of Chl *a* fluorescence (light state 1 – light state 2 transition; both thylakoid membrane reporters); (b) the hyper-osmotically induced quenching of Chl *a* fluorescence (a plasma membrane reporter); and (c) the electric conductivity of cell sus-

pensions (also a plasma membrane reporter) at various temperatures. We observed a reversible thermotropic transition between 19 °C and 22 °C (midpoint at 20.5 °C) in thylakoid membranes of mutant cells, but not of WT cells. Above 20.5 °C, WT and mutant cells were nearly indistinguishable; below 20.5 °C they differed conspicuously. On the other hand, the plasma membranes of both strains did not experience phase transitions and preserved selective permeability and osmotic functionality down to 2 °C. Fluorescence spectra at 77 K indicate that hyper-osmotic conditions and dark acclimation suppress the ratio of PS II fluorescence to PS I fluorescence in *Synechocystis* cells.

Materials and methods

Cultures of cyanobacteria

WT cells and desA$^-$/desD$^-$ mutant cells of *Synechocystis* sp. PCC 6803 (Tasaka et al. 1996) were cultured in the BG11 medium of Rippka et al. (1979) at 34 °C. The culture medium was buffered at pH 7.5 with 20 mM Hepes NaOH (hereafter buffered BG11). Cultures were aerated with air containing 1% (v/v) CO_2 and illuminated with white light at approx. 50 μmol m^{-2} s^{-1}.

Chl *a* concentrations of cyanobacterial cell suspensions were determined in N, N-dimethylformamide extracts (Moran 1982).

Solutions of defined osmolality

Solutions of defined osmolality were prepared by dissolving sorbitol or glycine betaine in buffered BG11. Osmolalities were measured with a cryoscopic osmometer (Osmomat 030, Gonotech, Berlin, Germany). The osmolality of buffered BG11 was 0.080 Osm kg^{-1}. The osmolality of a cell suspension equals the osmolality of the suspension medium since, practically, cells make no colligative contributions.

Cell suspensions are designated *hyper-osmotic* or *hypo-osmotic* relative to the *turgor threshold* of cells. The turgor threshold is defined as the maximal suspension osmolality at which cells still retain turgor, and in cyanobacterial cells it can be estimated by plotting the light-induced increase in Chl *a* fluorescence against the reciprocal of cell suspension osmolality (Papageorgiou and Alygizaki-Zorba 1997; Papageorgiou et al 1998). Only impermeable solutes contribute to the external osmolality that cells perceive. Turgor thresholds for WT cells and *desA$^-$/desD$^-$* mutant cells were 0.240–0.260 Osm kg^{-1} as determined fluorometrically at 34 °C.

Kinetics of Chl a *fluorescence*

Kinetics of light-induced and osmotically induced changes in Chl *a* fluorescence were measured with a PAM fluorometer (Heinz Walz, Effeltrich, Germany) as described by Papageorgiou and Alygizaki-Zorba (1997) and Papageorgiou et al. (1998). Samples were made in buffered BG11 at 10–12 μg Chl/ml and contained 20 μM DCMU. We used (a) for measuring fluorescence yields weak exciting light obtained as a train of periodic pulses (1.6 kHz, 1 μs wide, 650 nm, $\Delta\lambda$ = 25 nm, 70 nmol m^{-2} s^{-1}), and (b) for causing actinic effects, stronger continuous light ($\lambda > 520$ nm; 270 nmol m^{-2} s^{-1}) obtained from an incandescent lamp (KL-1500 Electronic, Schott Glasswerke, Wiesbaden, Germany). The fluorescence, which the weak modulated light excited, was amplified by a frequency-sensitive amplifier and was displayed by the Fluorescence Induction Program (Q_A Data Oy, Finland; see Tyystjaervi and Karunen, 1990) or the DA-100 software (Heinz Walz, Effeltrich, Germany). Only modulated fluorescence that was synchronous with measuring light was amplified and displayed.

Fluorescence spectra at 77 K

Fluorescence spectra at 77 K of *Synechocystis* cell suspensions were measured with a Hitachi 850 spectrofluorometer. Fluorescence was collected from the front surface of a flat plastic sample holder (thickness 2 mm). The excitation wavelength was 580 nm (10 nm bandwidth, exciting mainly phycobilins in PS II). Fluorescence spectra were scanned from 620 to 770 nm (1 nm bandwidth, scanning speed 40 nm s^{-1}, response time 2 s).

Chilling-induced damage of cyanobacterial cell membranes

The possibility of chilling-induced damage, that would allow cytoplasmic electrolytes to leak through the plasma membrane, was investigated by measuring the electric conductivity of cell suspensions, as described by Murata et al. (1984). Twice-washed cells were resuspended in milli-Q water at 100 μgChl/ml. Conductivity was monitored continuously with an electric conductivity meter (Model cm-2A; Toa Electronic Co., Tokyo, Japan) during cooling-rewarming cycles at a rate of 0.5 °C min^{-1}.

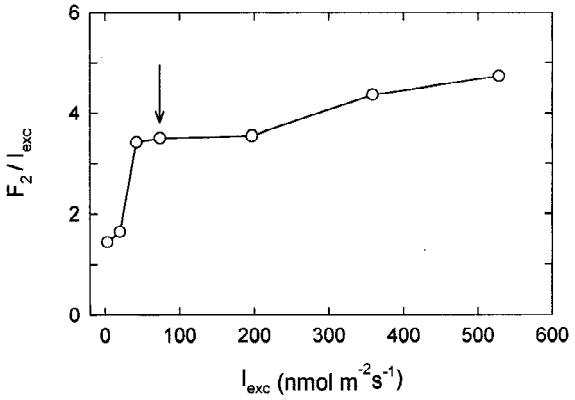

Figure 1. The ratio F_2/I_{exc} (proportional to the quantum yield of Chl *a* fluorescence at level F_2) as a function of I_{exc}, the modulated excitation intensity. Measurements with $desA^-/desD^-$ mutant *Synechocystis* sp. PCC 6803 cells. To overcome light meter limitations at very weak light, excitation intensities were measured with 100 kHz-modulated light, which is 62.5 times more intense than the 1.6 kHz-light exciting light. The arrow indicates the excitation intensity used in the experiments.

Results

Light/dark-induced and osmotically-induced changes of Chl a fluorescence

To ascertain that the modulated excitation sufficed to reduce Q_A to Q_A^- quantitatively in DCMU-treated *Synechocystis*, we plotted F_2/I_{exc} (a quantity proportional to the quantum yield of F_2-level fluorescence) against I_{exc}, the modulated excitation intensity (Figure 1). In the excitation intensity range from 50 to 200 nmol m^{-2} s^{-1} the yield of F_2 fluorescence was independent of I_{exc}. Above 200 nmol m^{-2} s^{-1} light acclimation of the cell led to the increase of Chl *a* fluorescence.

Figure 2 illustrates the two methods we employed in this research. In the *osmotic equilibration method* (Figure 2A; see Papageorgiou and Alygizaki-Zorba 1997) cyanobacterial cells were suspended either in hypo-osmotic medium (Osm = 0.080 kg^{-1}; trace I) or in hyper-osmotic medium (Osm = 0.487 kg^{-1}; trace II) and were dark-acclimated for 5 min before recording the fluorescence trace. Illumination with measuring light generated the characteristic weak Chl *a* fluorescence (level F_2, measured up to 30 s) of dark-acclimated cells (light state 2; Mohanty and Govindjee 1973; Fork et al. 1979; Dominy and Williams 1987). At the F_2 level all Q_A was reduced to Q_A^- (see Figure 1). Subsequent actinic illumination drove Chl *a* fluorescence to the higher level F_1 that characterizes light-acclimated (light state 1) cells. After the actinic light was turned off, cells changed back to the dark-acclimated state (F_2). After the measuring light was turned off, the fluorescence signal vanished. The fluorescence difference $\Delta F = F_1 - F_2$ was smaller in the hyper-osmotic suspension (Figure 2A, trace II) than in the hypo-osmotic suspension (Figure 2A, trace I). Plots of $\Delta F/F_2$ against inverse osmolality (kg Osm^{-1}) are linear, indicating ideal osmotic behavior of cyanobacterial cells, and allow determination of turgor thresholds (Papageorgiou and Alygizaki-Zorba, 1997).

In the *hyper-osmotic injection method* (Figure 2B; Papageorgiou et al. 1998) a suspension of *Synechocystis* cells in buffered BG11 (0.080 Osm kg^{-1}) was first brought to the light-acclimated state (fluorescence level F_1, measured up to 30 s or more) and then it was mixed either with buffered BG11 (ΔOsm kg^{-1} = 0; Figure 2B, trace III) or with sorbitol-containing buffered BG11 (ΔOsm kg^{-1} = 0.407; Figure 2B, trace IV). Mixing was performed by microsyringe injection and was completed within 10 ms. This time limit is set by the rate of signal sampling of the PAM fluorometer. The injection depressed Chl *a* fluorescence to the lower level denoted as F_1' (measured up to 30 s or more) either because of sample dilution (trace III) or because of the combined effects of sample dilution and of hyper-osmotic quenching (trace IV). When the actinic light was turned off, Chl *a* fluorescence decayed to the F_2' level, which was below the F_2 level of dark acclimated cells because of sample dilution. The difference $\Delta F' = F_1' - F_2'$, was independent of dilution and smaller after hyper-osmotic injection (Figure 2B, trace IV) than after hypo-osmotic injection (Figure 2B, trace III). Plots of $\Delta F'/F_2'$ against reciprocal osmolality allow estimation of turgor thresholds of cyanobacterial cells. Additionally, the injection method allows monitoring of dynamic solute and water fluxes across plasma membranes of cyanobacterial cells (Papageorgiou et al. 1998).

Light acclimation of wild-type and mutant Synechocystis cells at chilling temperatures

According to Fork et al. (1979) and Fork and Satoh (1983) thylakoid membranes must be fluid in order to allow for dark- or light-acclimative transitions of cyanobacterial cells. Membrane fluidity depends on fatty acid unsaturation and can be manipulated physiologically by controlling the culture temperature, or genetically, by inactivating membrane-bound acyl-lipid

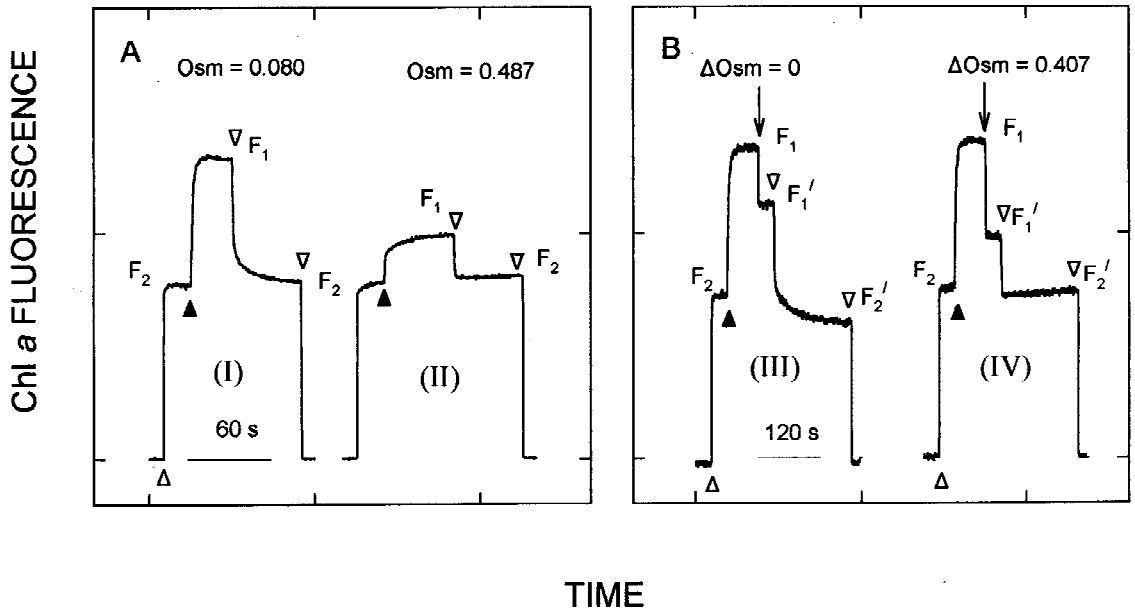

Figure 2. Light-induced and osmotically induced changes in Chl *a* fluorescence intensity in suspensions of WT *Synechocystis* sp. PCC 6803 cells. Open triangles: on/off times of modulated exciting light. Solid triangles: on/off times of continuous actinic light. Cell suspensions were treated with 20 μM DCMU and were acclimated to darkness for 5 min prior to recording the kinetic traces. Time bars indicate time scales. A. Fluorescence kinetics recorded with cells suspended in buffered BG11 (0.080 Osm kg^{-1}; trace I) or in buffered BG11 that was supplemented with 0.384 M sorbitol (ΔOsm = 0.407 kg^{-1}; trace II). B. Fluorescence kinetics recorded with cells suspended in buffered BG11. At times indicated by arrows either buffered BG11 (ΔOsm = 0 kg^{-1}; trace III), or sorbitol in buffered BG11 (ΔC = 0.383 M; ΔOsm = 0.407 kg^{-1}; trace IV) were injected. Cell suspension dilution 1:4, v/v.

desaturases (Murata and Los 1997 and cited references). The transition from light acclimation to dark acclimation was impaired below 44 °C, when thermophilic *Synechococcus lividus* cells were cultured at 55 °C, and below 37 °C when they were cultured at 38 °C (Fork et al. 1979). Similar results were reported for the dark-acclimation to light-acclimation transition of *S. lividus* (Fork and Satoh 1983).

In the experiment of Figure 3 we recorded light-induced and dark-induced kinetics of Chl *a* fluorescence with cell suspensions equilibrated at different temperatures. Sharply different behaviors are evident between WT and mutant cells. In WT cells the light-induced fluorescence rise (F_2 to F_1) became progressively slower as cell suspension temperature decreased from 30 °C to 6 °C. At the same time, the dark-induced fluorescence decay (F_1 to F_2) became also slower and clearly biphasic at 6 °C (Figure 3A). However, the fluorescence difference between the light-acclimated state and the dark-acclimated state, $\Delta F = F_1 - F_2$, remained constant. In mutant cells the fluorescence kinetics at 6 °C and 19 °C were entirely different from those at 26 °C and 30 °C: level F_1 was suppressed and level F_2 raised, indicating diminishing ability of the chilled cells for the light acclimative transition. At 30 °C or at 26 °C, however, WT cells and *desA*$^-$*/desD*$^-$ mutant cells were indistinguishable.

Figure 4 plots ΔF values (obtained as in Figure 3) against the cell suspension temperature. In WT cells ΔF declined slightly without interruption. In contrast, in mutant cells ΔF dropped abruptly to a lower level between 22 °C and 19 °C (midpoint of 20.5 °C) and approached zero as sample temperature approached 0 °C. The discontinuity in the temperature curve of ΔF indicates a physical state transition that occurred in the thylakoid membranes of mutant cells but not of WT cells. The transition was fully reversible. As soon as the chilled mutant cells were rewarmed to 34 °C normal light-induced and dark-induced kinetics of Chl *a* fluorescence were restored (data not shown).

Chilling treatment and plasma membrane functionality

Figure 5 displays temperature plots of electric conductivities of cell suspensions from 26 °C to 2 °C, measured continuously during cooling and rewarming cycles. There were no discontinuities evident that

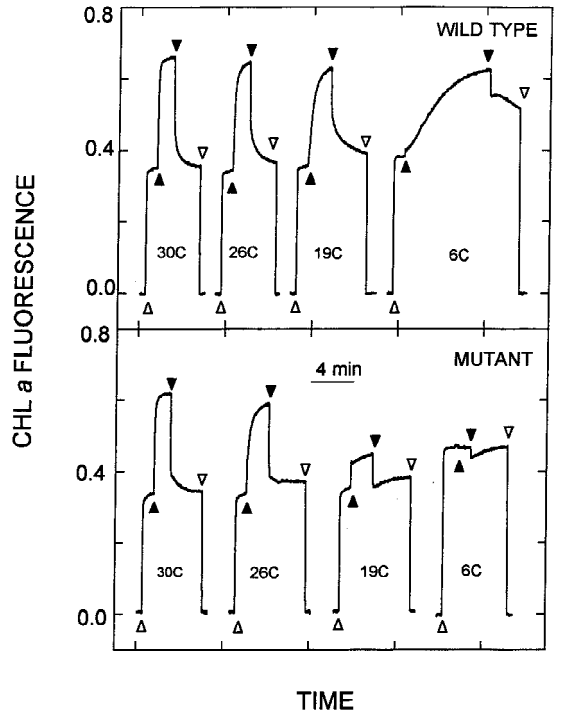

Figure 3. Light-induced changes of Chl *a* fluorescence intensity recorded with WT cells (upper panel) and *desA*$^{-}$*/desD*$^{-}$ mutant cells (lower panel) of *Synechocystis* sp. PCC 6803 after dark acclimation and equilibration at indicated temperatures. Cells were suspended in buffered BG11 in the presence of 20 μM DCMU. A time bar indicates the time scale.

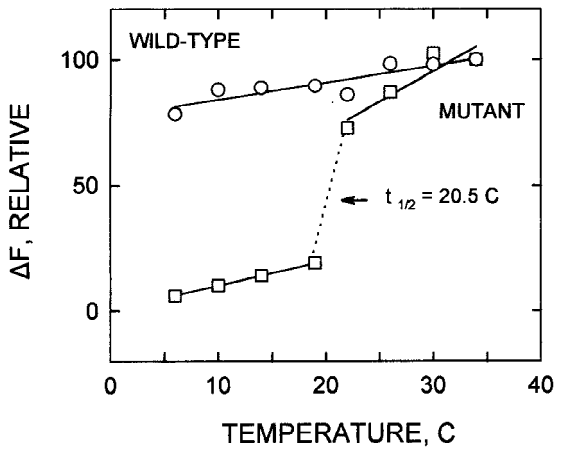

Figure 4. The difference ($\Delta F = F_1 - F_2$) between the Chl *a* fluorescence intensity cells in the light-acclimated state (F_1) and in the dark acclimated state (F_2) as a function of the assay temperature. WT cells and *desA*$^{-}$*/desD*$^{-}$ mutant *Synechocystis* sp. PCC 6803 cells were suspended in buffered BG11, in the presence of 20 μM DCMU.

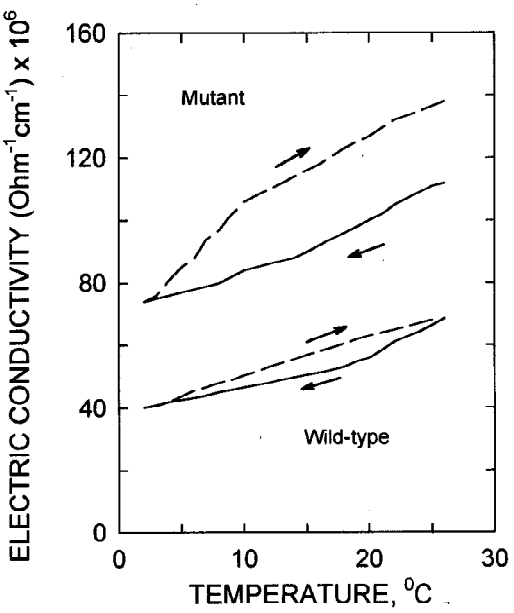

Figure 5. Temperature dependence of the electric conductivity of suspensions of wild-type cells and *desA*$^{-}$*/desD*$^{-}$ mutant cells of *Synechocystis* sp. PCC 6803 during temperature scans from 26 °C to 2 °C (solid lines) and back (2 °C to 26 °C; dashed lines). The cells were suspended in milli-Q water at 100 μg Chl *a* ml^{-1} and the temperature was scanned at a constant rate of 0.5 °C min^{-1}. Arrows indicate the directions of temperature scanning.

might suggest uncontrollable leakage of cytoplasmic electrolytes. A discontinuous irreversible increase in electric conductivity would signify a phase transition of plasma membranes and, indeed, such an effect was detected upon chilling *Anacystis nidulans* cells but not upon chilling *Anabaena variabilis* cells (Murata et al. 1984). Thus, although the *desA*$^{-}$*/desD*$^{-}$ mutant of *Synechocystis* sp. PCC 6803 resembles the chilling-sensitive *A. nidulans* in glycerolipid unsaturation, its plasma membranes behave similarly to those of the chilling-tolerant *A. variabilis.* For unknown reasons suspensions of mutant cells were more conductive than suspensions of WT cells.

Figures 6 and 7 illustrate an application of the hyper-osmotic injection method: comparison of WT and mutant cells, at 34 °C and at 14 °C, with respect to permeabilities to sorbitol, glycine betaine and NaCl. Cells were first equilibrated to the temperature of the assay, then acclimated to light and given hyper-osmotic shocks of 1.0 Osm kg^{-1}. All three solutes caused cell volume contraction as reported by fluorescence quenching. Cell contraction at hyper-osmotic conditions witnesses an osmotically functional plasma membrane that is freely permeable to water but imper-

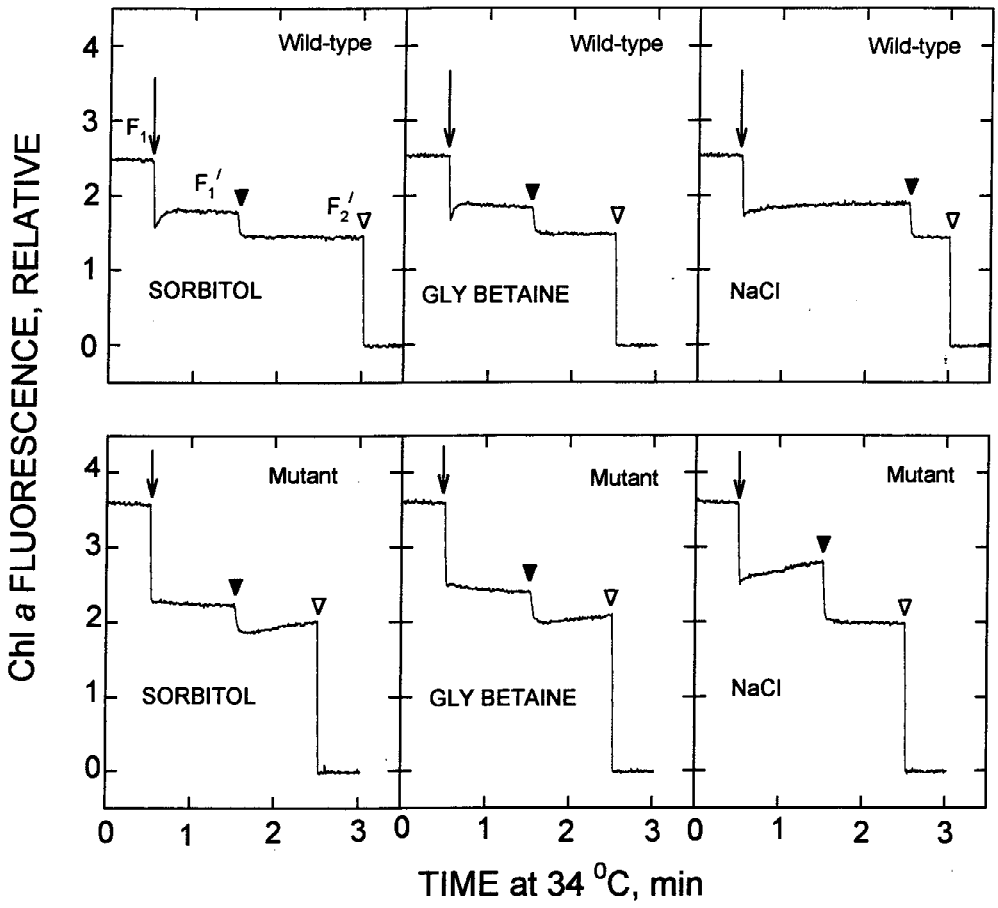

Figure 6. Chl *a* fluorescence kinetics recorded with WT and *desA*$^{-}$/*desD*$^{-}$ mutant cells of *Synechocystis* sp. PCC 6803 at 34 °C after 1.0 Osm kg^{-1} hyper-osmotic shocks with sorbitol, glycine betaine and NaCl solutions of defined osmolality. Details and symbols as in Figure 2B.

meable to the tested solutes (in the time frame of the experiment). In addition, a characteristic quenching overshoot was observed after addition of sorbitol, or glycine betaine, to suspensions of WT cells at 34 °C (Figure 6, top left and middle panels). Fluorescence recovery, after the quenching overshoot, was partial and occurred with half times 2–4 s. The quenching overshoot was not observed with WT cells at 14 °C (Figure 7 top, left and middle panels), nor with mutant cells either at 34 °C (Figure 6 bottom, left and middle panels) or at 14 °C (Figure 7 bottom, left and middle panels). These results indicate that, although plasma membranes of mutant cells are more rigid than those of WT cells, as they contain no polyunsaturated fatty acids, mutant and WT cells respond to osmotic upshocks indistinguishably. This stands in sharp contrast to the behavior of thylakoid membranes: at 14 °C thylakoid membranes of mutant cells, but not of WT cells, had already experienced a physical state transition (Figure 4).

The case of NaCl merits special attention. The hyper-osmotic jump, after the injection of NaCl, causes rapid efflux of cytoplasmic water, cell contraction (Blumwald et al. 1984) and depresses Chl *a* fluorescence ($t_{1/2} < 10$ ms; Figures 6 and 7). These initial events were followed by a slow rise of fluorescence, as NaCl and water started moving into the cells. This fluorescence rise is much slower in *Synechocystis* sp. PCC 6803 cells than in *Synechococcus* sp. PCC 7942 cells (time constant $t_{1/2} \approx 200$ ms; Papageorgiou et al. 1998). Apparently, the permeability of *Synechocystis* to NaCl is much lower than the permeability of *Synechococcus* and this may be a contributing factor for the higher salinity tolerance of the former species.

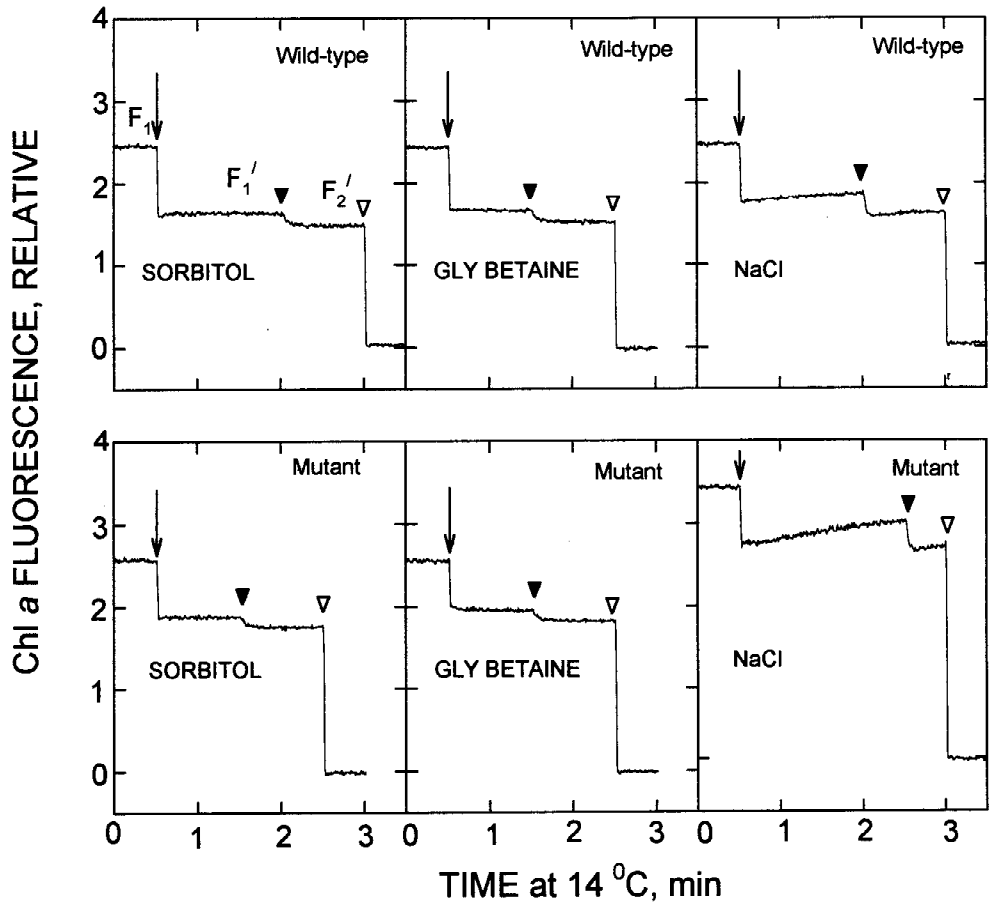

Figure 7. Chl *a* fluorescence kinetics recorded with WT cells and *desA*$^{-}$/*desD*$^{-}$ mutant cells of *Synechocystis* sp. PCC 6803 at 14 °C after 1.0 Osm kg^{-1} hyper-osmotic shocks with sorbitol, glycine betaine and NaCl solutions of defined osmolality. Details and symbols as in Figure 1B.

Osmotically induced redistribution of Chl a *excitation*

Light-acclimated cyanobacterial cells cannot sustain the high yield of Chl *a* fluorescence if the cell suspension is made hyper-osmotic (Papageorgiou et al. 1998). Conversely, hyper-osmotic conditions prevent the light-induced rise of Chl *a* fluorescence in dark-acclimated cyanobacterial cells (Papageorgiou and Alygizaki-Zorba 1997). With respect to Chl *a* fluorescence at room temperature, light-acclimated cyanobacterial cells under hyper-osmotic conditions resemble hypo-, or hyper-osmotic suspensions of dark-acclimated cells. The question is whether hyper-osmotic conditions suppress the PS II fluorescence and enhance the PS I fluorescence of light-acclimated cyanobacterial cells at low temperature, just as dark acclimation does.

Figure 8 displays fluorescence spectra of WT *Synechocystis* cells recorded at 77 K with 580 nm excitation. The spectra were corrected numerically (every 0.1 nm) by reference to the emission of a standard lamp of known temperature (295 K). Cells suspended in buffered BG11 medium (hypo-osmotic suspension; 0.08 Osm kg^{-1}) were first acclimated to light, as in Figure 2A, and then they were frozen in liquid nitrogen, as quickly as possible, either as such, or after a hyper-osmotic jump with sorbitol-containing medium (hyper-osmotic suspension; ΔOsm kg^{-1} 0.91). The spectra are normalized at 724 nm. According to the difference spectrum, in hypo-osmotic suspension light-acclimated cells emitted more fluorescence at 666 nm (allo-phycocyanin), 685 nm (CP43) and 696 nm (CP47; see Papageorgiou 1996 and cited references for band maxima assignments) than in hyper-osmotic cell suspension.

Light acclimative transitions in cyanobacteria and other oxygenic photosynthetic organisms cause opposing changes in the 77 K fluorescence emission

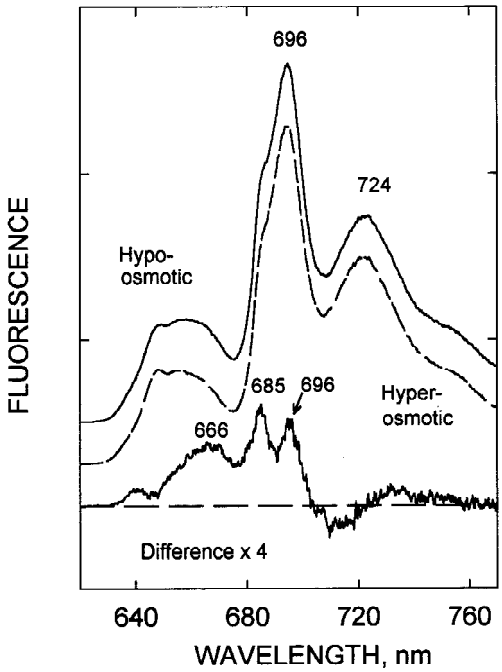

Figure 8. Fluorescence spectra at 77 K of WT *Synechocystis* sp. PCC 6803 cells recorded with excitation at 580 nm. The spectra are corrected for the spectral response of emission monochromator/photomultiplier. Light-acclimated cells in buffered BG11 were frozen in liquid nitrogen either before (hypo-osmotic suspension; solid line) or after osmotic upshock (ΔOsm kg^{-1} = 0.91; hyper-osmotic suspension, dashed line). The displayed spectra are normalized at 724 nm and shifted in the ordinate for clarity. Difference: fluorescence spectrum of frozen hypo-osmotic cell suspension minus the fluorescence spectrum of frozen hyper-osmotic cell suspension.

of PS II and PS I (Delphin et al. 1996; Murata 1969). Light-acclimated cells emit relatively more PS II fluorescence (F_{684}; F_{696}) and relatively less PS I fluorescence ($F_{720-730}$). Thus, the $(F_{696} - F_{720})/F_{720}$ fluorescence ratio at 77 K may serve to diagnose the state of light or dark acclimation of photosynthetic cells. The ratio measures essentially the excess Chl *a* excitation residing in PSII relative to that residing in PS I. According to Table 1, the $(F_{696} - F_{720})/F_{720}$ ratio was lower when light-acclimated cells existed in hyper-osmotic suspension than when they existed in hypo-osmotic suspension. In hyper-osmotic suspension, the fluorescence ratio of light-acclimated cells approached that of the dark-acclimated cells, regardless of whether the latter were in hypo-, or in hyper-osmotic suspension. These results show that PS I chlorophylls received excess excitation (a) when the cyanobacterial cells were in the dark-acclimated state, and (b) when light-acclimated cells experienced hyper-osmotic conditions.

Discussion

Biological membranes are more fluid when glycerolipids are rich in unsaturated fatty acids. Although membrane fluidity (inverse of viscosity) is a tenuous term, it nevertheless imparts a clear notion of dynamic molecular movements within the membrane phase (see Murata and Los 1996 and cited literature). It follows that molecular movements must be more facile in membranes of WT *Synechocystis* cells, which contain polyunsaturated fatty acids, than in membranes of *desA*$^-$/*desD*$^-$ mutant cells, which lack them (Tasaka et al. 1996). In the present paper we probed fluidity-dependent properties of thylakoid and plasma membranes in intact *Synechocystis* sp. PCC 6803 cells with Chl *a* fluorescence.

We used Chl *a* fluorescence in two ways: First, we examined its changes during dark-light-dark acclimative cycles (light state transitions) and second after osmolality upshifts in cell suspensions. Dark-light-dark acclimative transitions in cyanobacterial cells involve spatial displacements and associations/dissociations of integral thylakoid membrane holochromes that presumably affect excitation energy coupling to phycobilisomes (Fork et al. 1979; Fork and Satoh 1983; Mullineaux 1994; Bald et al. 1996). Chl *a* fluorescence changes, therefore, that occur during such transitions depend on movements of proteins in thylakoid membranes. In contrast, osmolality upshifts or downshifts in cell suspensions generate water and solute fluxes through the selectively permeable plasma membrane. In this case, Chl *a* fluorescence changes witness the functional integrity of plasma membranes. (Papageorgiou and Alygizaki-Zorba 1997; Papageorgiou et al. 1998).

When cyanobacterial cells are cooled, thylakoid membrane lipids shift to the phase separated state ahead of plasma membrane lipids (Murata 1989). Since the inactivation of acyl-lipid desaturases increases fatty-acid saturation in both membranes (Murata and Nishida, 1995), we expected: (a) membranes to be more susceptible to chilling-induced damage in *desA*$^-$/*desD*$^-$ mutant cells than in WT cells; and (b) thylakoid membranes of both strains to be more susceptible to chilling-induced damage than plasma membranes. Both expectations proved to be correct.

Table 1. Redistribution of electronic excitation between PS II and PS I chlorophylls in *Synechocystis* sp. PCC 6803 as a result of acclimation (a) to light or darkness and (b) to hypo- or hyper-osmotic conditions. In brackets, per cent values

Treatments	$(F_{696} - F_{720}) / F_{720}$
Acclimation to darkness[a]	100
Acclimation to light[b]	115 ± 3
Acclimation to light followed by hyper-osmotic injection[c]	104 ± 4
Acclimation to light followed by hyper-osmotic injection and acclimation to darkness[d]	97 ± 1

Cell suspensions were light/dark acclimated and osmotically acclimated at room temperature and then frozen in liquid nitrogen (77 K) to record fluorescence spectra excited at 580 nm.
[a]*Dark acclimated cells*: Darkness 5 min, measuring light illumination (30 s; 650 nm. 70 nmol m^{-2} s^{-1}); frozen to 77 K.
[b]*Light acclimated cells*: Darkness 5 min, measuring light (30 s), actinic light (60 s); frozen to 77 K.
[c]*Hyperosmotically shocked light acclimated cells*: Darkness 5 min, measuring light (30 s; 650 nm. 70 nmol m^{-2} s^{-1}), actinic light (60 s) hyperosmotic injection (ΔOsm kg^{-1} = 0.91); frozen to 77 K.
[d]*Hyperosmotically shocked dark acclimated cells*: Darkness 5 min, measuring light (30 s; 650 nm. 70 nmol m^{-2} s^{-1}), actinic light (60 s), hyperosmotic injection (ΔOsm kg^{-1} = 0.91), measuring light (120 s); frozen to 77 K. Values are averages of three independent experiments.

Thylakoid membranes of mutant cells experienced a reversible physical state change between 22 °C and 19 °C (midpoint at 20.5 °C; Figure 4); thylakoid membranes of WT cells did not. The variant behavior of mutant cells must reflect the absence of polyunsaturated fatty acids that makes their thylakoid membranes more susceptible to thermotropic transitions. Zakim et al. (1992) reported that the fluidity of lipid bilayers drops about 10-fold on going from the liquid-crystalline state to the gel state. The discontinuity in the temperature curve of ΔF in mutant cells (Figure 4) may pertain to a similar fluidity drop of thylakoid membrane lipids. Conversely, the absence of a discontinuity in the temperature curves of ΔF WT cells may suggest that thylakoid membranes are in the liquid-crystalline-state in the 34 °C to 6 °C temperature range. The more fluid thylakoid membranes of WT cells allow movements of integral membrane proteins and thus they enable light/dark-induced Chl *a* fluorescence changes. In *desA*$^-$*/desD*$^-$ mutant cells such protein movements are impeded below the phase transition temperature (20.5 °C) as thylakoid membranes become more rigid.

In contrast to the characteristically different temperature responses of thylakoid membranes, we detected no conspicuous differences in the plasma membranes of WT and *desA*$^-$*/desD*$^-$ mutant cells of *Synechocystis* sp. PCC 6803. Both strains responded to hyper-osmotic shocks similarly, either at 34 °C (Figure 6) or at 14 °C (Figure 7), and there were no discontinuities in the temperature curves of electric conductivities of cell suspensions that might indicate leakage of cytoplasmic ions (Figure 5). Plasma membranes of mutant cells maintained functionality and were indistinguishable from plasma membranes of WT cells in the 34 °C to 6 °C temperature range, except for one particular effect: the hyper-osmotic fluorescence quenching overshoot which was exhibited only by WT cells and only at 34 °C (Figures 6 and 7). This effect probably relates to a transient cell volume contraction. It occurred under conditions conducive to plasma membrane fluidity, i.e. presence of polyunsaturated fatty acids and normal temperatures, but not at unfavorable conditions for membrane fluidity, i.e. absence of polyunsaturated fatty acids and chilling temperature. The hyperosmotic fluorescence quenching overshoot may constitute a marker of plasma membrane fluidity in intact cyanobacterial cells.

According to Figure 8 and Table 1, hyperosmotic conditions and dark acclimation had similar effects on Chl *a* fluorescence: suppression of the ratio of PS II

fluorescence to PS I fluorescence. The question is if this indicates a common underlying physical cause for both phenomena. In cyanobacteria, the dark acclimative transition is triggered by the reduction of intersystems redox intermediates with electrons provided by endogenous low potential substrates (Dominy and Williams 1987). Its end result is the increase in the absorption cross section of PS I and the decrease of the absorption cross section of PS II (Mullineaux and Holzwarth 1991). A possible way to change the absorption cross sections of PS I and PS II is to modify their connectivities to phycobilisomes (see models by Mullineaux 1994; Bald et al 1996).

Depletion of cytoplasmic water under hyper-osmotic conditions may enhance the connectivity of phycobilisomes to PS I and enlarge its absorption cross section. Macroscopically, this would appear as quenching of the room temperature Chl *a* fluorescence of light-acclimated cells. The sensitivity of the phycobilisome-to-PS I excitation transfer route to hyper-osmotic conditions is supported by a recent report, according to which treatment of cyanobacterial cells with N-ethyl maleimide (a blocker of this route; Glazer et al. 1994) makes Chl *a* fluorescence of light-acclimated cells insensitive to hyper-osmotic shocks (Stamatakis and Papageorgiou 1999). In contrast, the Chl *a* fluorescence of light-acclimated cells of the psaA/psaB-deletion mutant of *Synechocystis* sp. PCC 6803 (Shen et al. 1993) was sensitive to hyper-osmotic conditions (R. Govindjee, Govindjee, G.C. Papageorgiou and N. Murata, unpublished experiment). This probably indicates that other Chl *a*-containing PS I proteins, besides the psaA/psaB dimer, may serve as sinks of phycobilisome excitation in the psaA/psaB-deletion mutant.

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