Photosystem II fluorescence quenching in the cyanobacterium *Synechocystis* PCC 6803: involvement of two different mechanisms

K. El Bissati [a], E. Delphin [a], N. Murata [b], A.-L. Etienne [a], D. Kirilovsky [a,*]

[a] *URA 1810, CNRS, ENS, 46 rue d'Ulm, 75230 Paris, France*
[b] *Department of Regulation Biology, National Institute for Basic Biology, Myodiji, Okazaki 444 Japan*

Abstract

The structural changes associated to non-photochemical quenching in cyanobacteria is still a matter of discussion. The role of phycobilisome and/or photosystem mobility in this mechanism is a point of interest to be elucidated. Changes in photosystem II fluorescence induced by different quality of illumination (state transitions) or by strong light were characterized at different temperatures in wild-type and mutant cells, that lacked polyunsaturated fatty acids, of the cyanobacterium *Synechocystis* PCC 6803. The amplitude and the rate of state transitions decreased by lowering temperature in both strains. Our results support the hypothesis that a movement of membrane complexes and/or changes in the oligomerization state of these complexes are involved in the mechanism of state transitions. The quenching induced by strong blue light which was not associated to D1 damage and photoinhibition, did not depend on temperature or on the membrane state. Thus, the mechanism involved in the formation of this type of quenching seems to be unrelated to the movement of membrane complexes. Our results strongly support the idea that the mechanism involved in the fluorescence quenching induced by light 2 is different from that involved in strong blue light induced quenching. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

In higher plants, algae and cyanobacteria, the energy absorption occurs at the level of the antenna of two membrane-pigment complexes, the Photosystem I (PS I) and the Photosystem II (PS II). The absorbed energy is transferred to the reaction centers, operating in series. The photochemical reaction is the major pathway of deactivation of the excitons formed by the absorption of photons by the antenna of PS I and PS II (more than 90% of absorbed light energy). Fluorescence and thermal dissipation are the other ways to deactivate these excitons; fluorescence representing only 0.3–3% of the absorbed light. There is an inverse relationship between photochemistry and fluorescence emission. Photochemistry and the related photochemical quenching of fluorescence (q_P) are maximal when all PS II centers are open and then the fluorescence yield is low. When all centers are closed, q_P is suppressed and the fluorescence yield is concomitantly increased to its maximal level. In other terms, the fluorescence is modulated by the oxidoreduction state of the primary acceptor Q_A [1,2]. When Q_A is oxidized, a minimal level of fluo-

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* Corresponding author. Fax: +33-1-44 32 3935;
E-mail: kirilov@wotan.ens.fr

rescence is observed while the fluorescence reaches a maximum level when Q_A is fully reduced.

Non-photochemical processes can also reduce the yield of fluorescence (non-photochemical PS II fluorescence quenching, NPQ) (for reviews see [3–5]). Three major physiological processes contribute to total NPQ: state transitions, q_E and photoinhibition. The state transitions involve the redistribution of the absorbed energy between PS II and PS I by altering the antenna size of PS II and/or spillover to PS I. The energy-dependent quenching (q_E) is related to a transthylakoid proton gradient formed during electron transport. High light intensities induce PS II fluorescence quenching and an irreversible inactivation of PS II (photoinhibition; for reviews see [6,7]). This phenomenon is paralleled by the degradation of the D1 protein, an essential constituent of the PS II. Recovery of variable fluorescence and oxygen evolving activity needs replacement of the damaged D1 protein.

The mechanism that regulates the redistribution of energy between PS II and PS I, known as state transitions, was first detected by Murata [8,9] and Bonaventura and Myers [10]. They showed that exposure of algae to light absorbed predominantly by PS II (light 2) causes a relative decrease of the PS II fluorescence yield. Inversely, illumination with light absorbed preferentially by PS I (light 1) induces a relative increase of the PS II fluorescence yield. In green algae and higher plants the mechanism of state transitions involves redox-dependent phosphorylation/dephosphorylation reactions of the PS II antenna, the light harvesting complex II (LHCII), inducing a movement of part of the LHCII between PS II and PS I (reviewed by [11–13]).

The mechanisms involved in state transitions in red algae and in cyanobacteria, which contain phycobilisomes as PS II antenna, are not as well characterized as in higher plants. Two main models have been initially proposed to explain the way in which the energy is redistributed between PS II and PS I in state transitions: (1) redistribution of the absorbed energy by changes in the spillover between PS II and PS I [14–17] or (2) changes in the cross-section of the antenna of both PS I and PS II [18,19]. The results of Mullineaux and co-workers support the hypothesis that a lateral diffusion of the phycobilisomes leads to changes in the cross-section of PS II and PS I

antennae [20–23]. However, this hypothesis remains controversial. Results from other laboratories have suggested that the mobile elements are the photosystems (PS I and/or PS II) [16,24,25].

Near-saturating and saturating intensities of white light induce NPQ in higher plants, eukaryotic algae (green, brown and red) and cyanobacteria. In higher plants and green and brown algae, at least part of this quenching is dominated by a mechanism involving an increase in the thermal dissipation of absorbed energy in the PS II antenna complex induced by the transthylakoid proton gradient formed during electron transport [26–28]. The formation of this quenching is accompanied by the accumulation of deepoxidated xanthophyll [29–32]) and conformational changes (most probably, aggregation) of LHCII [33–37]. In the red algae *Rhodella violacea* and *Porphiridium cruentum*, near-saturating white light illumination also induces a large ΔpH-dependent quenching [38,39]. The molecular mechanism involved in this type of quenching in red algae, which does not have LHCII or the carotenoid cycle, remains to be elucidated. Campbell et al. [40] suggested that in cyanobacteria, the PS II fluorescence quenching induced by high intensities of white light is a result of the state transition mechanism and/or photoinhibition. However, no unambiguous proof for this hypothesis has been yet provided.

The role of phycobilisome and/or photosystem mobility in state transitions and on NPQ in cyanobacteria is a point of interest to be elucidated. In the present work, we studied the influence of temperature and the physical state of the membrane on the fluorescence level changes induced by selective illumination of one of the two photosystems or by strong light in wild-type and *desA⁻/desD⁻* mutant cells of *Synechocystis* PCC 6803. *desA⁻/desD⁻* mutant cells do not contain polyunsaturated fatty acids presenting a modified membrane fluidity [41]. In those mutant cells, the membrane phase transition occurs at higher temperatures than in wild-type cells [41].

In this article, we first provide data supporting the idea that in cyanobacteria, the movement of membrane-complexes is involved in the state transition mechanism. Our results also indicate that a mechanism different from the state transition or photoinhibition is involved in the quenching of PS II fluorescence induced by strong light.

2. Materials and methods

2.1. Strain and culture conditions

The wild-type strain of *Synechocystis* sp. PCC 6803 was originally provided by Dr. J.G.K. Williams (DuPont de Nemours, Wilmington, DE, USA). The mutant (*desA⁻/desD⁻*) was obtained by targeted mutagenesis in the *desA* and *desD* genes [41]. First a *desA⁻* mutant was obtained as described by [42]. Then, the *desD* gene in *desA⁻* cells was inactivated to generate the *desA⁻/desD⁻* mutant [41]. The *desA* and *desD* genes encode acyl-lipid desaturases which introduce double bounds at the Δ 12 and Δ 6 positions, respectively, of C18 fatty acids. The mutation of each of these genes by insertion of an antibiotic resistance gene cartridge completely eliminated the corresponding desaturation reaction. Wild-type and mutant *desA⁻/desD⁻* cells were grown photoautotrophically in a BG11 medium, in a rotatory shaker at 30°C under 90 μmol photons m^{-2} s^{-1}, in a CO$_2$ enriched atmosphere. Both wild-type and mutant cells were harvested during exponential phase by centrifugation, washed and resuspended in buffered growth medium (0.5 M HEPES, pH 6.8) for fluorescence measurements.

2.2. Fluorescence measurements

The yield of chlorophyll (Chl) fluorescence was continuously monitored in a modulated fluorometer (PAM Chl fluorometer; Walz, Effelrich, Germany) adapted to a DW1 Hansatech oxygen electrode as previously described [43]. Cell suspensions (2 μg Chl ml^{-1}) were placed in a stirred cuvette (30°C). Cells adapted to darkness for 5 min were brought to state 1 by illumination with blue light (450 nm, Corning 4.96) at 20 μmol photons m^{-2} s^{-1} and to state 2 by illumination with orange light (601 nm, filter: Balzer B-40 599 10) at 12 μmol photons m^{-2} s^{-1}. See Fig. 1 for illustration of a fluorescence trace.

The minimal fluorescence level (F_0) was determined by illuminating dark-adapted cells with a low intensity of red-modulated light (pulses of 1 μs, 1.6 kHz, 0.024 μmol photons m^{-2} s^{-1}). The minimal fluorescence level in the light-adapted state (F_0') was determined by briefly interrupting the continuous blue or orange light. In cyanobacteria, the F_0 and
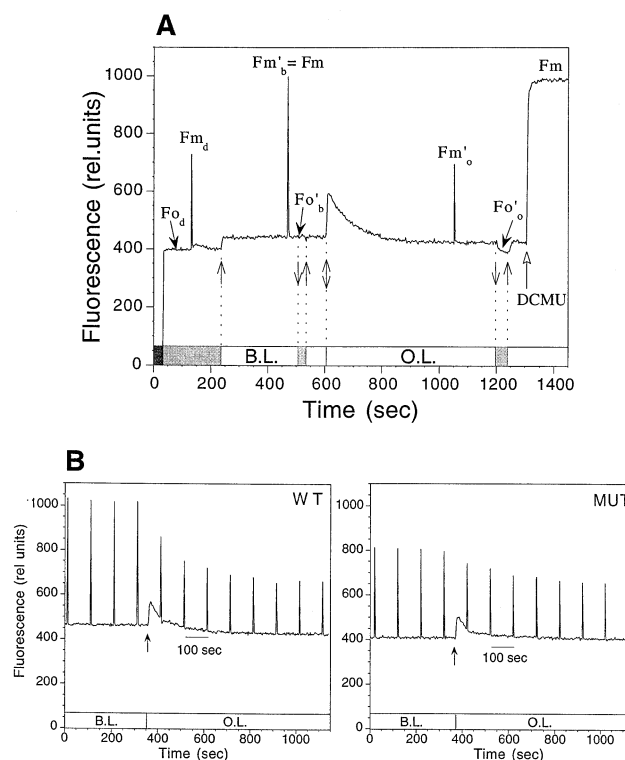

Fig. 1. Measurements of fluorescence yield by a PAM fluorometer during different types of illumination in wild-type cells. (A) Dark-adapted cells for 5 min (end of this period indicated by a dark label at the bottom of the figure) (under non actinic modulated light, M.L.) were successively illuminated with blue light (B.L.) at 20 μmol photons m^{-2} s^{-1} and orange light (O.L.) at 12 μmol photons m^{-2} s^{-1}. Saturating pulses (3200 μmol photons m^{-2} s^{-1}, 600 ms duration) were applied to assess F_m dark (F_{md}) and $F_{m'}$. Chl concentration was 2 μg ml^{-1} (same concentration in the following experiments). (B) State 1 to state 2 transition in wild-type (WT) and mutant cells (MUT). Dark-adapted cells (for 5 min) were illuminated successively with blue light (B.L.) at 20 μmol photons m^{-2} s^{-1} and orange light (O.L.) at 12 μmol photons m^{-2} s^{-1}. Saturating pulses separated by 100 s (3200 μmol photons m^{-2} s^{-1}, 600 ms duration) were applied to assess $F_{m'}$.

F_0' levels detected with a PAM fluorometer contain a contribution from phycocyanin fluorescence. As a consequence these levels depend on the PC/Chl ratio of the cells. We carried out the experiments using wild-type and mutant cells containing the same ratio of these pigments.

Maximum fluorescence level of the dark adapted (F_{md}) or light-adapted cells ($F_{m'}$) was measured by a 600 ms high-intensity white pulse (3200 μmol photons m^{-2} s^{-1}). Application of such pulses of intense

light, which transiently close all PS II centers and remove q_P, serves to distinguish q_P from NPQ at any time. The saturating multiple turnover white pulses were produced by an electronic shutter (Uniblitz, Vincent, USA, opening time of 2 ms) put in front of a KL-1500 quartz-iodine lamp (Schott, Mainz, Germany) and controlled by the accessory module PAM-103. The maximal level of fluorescence (F_m) cannot be determined in darkness, since in cyanobacteria, a large NPQ is present under dark conditions. As a consequence, F_m was determined in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (10 μM) and white or blue light. This inhibitor blocks the electron transport from Q_A^- to Q_B causing reaction center closure and cancels all q_P. The q_P was calculated using the equation of van Kooten and Snel [44] $q_P = (F_{m'} - F_s)/(F_{m'} - F_{0'})$, F_s = steady-state fluorescence level under illumination. The NPQ was quantified by using the Stern–Volmer formulation, NPQ $= (F_m - F_{m'})/F_{m'}$ (in [28]).

Fluorescence was measured at different temperatures (from 2 to 30°C). When fluorescence measurements were carried out at temperatures lower than 30°C, the lowering of the temperature was done in darkness, except when state 1 to state 2 transition was induced. In this case it was done under blue light illumination.

The fluorescence emission spectra at 77K were recorded on a Hitachi F-3010 Fluorescence spectrophotometer. Excitation was done at 605 nm. Emission was scanned from 620 to 800 nm. 1 ml of cell suspension (2 μg Chl ml^{-1}), brought to state 1 or state 2 by blue or orange light as for PAM measurements, was quickly filtered and the filter immediately plunged into liquid nitrogen.

2.3. Oxygen measurements

The amount of oxygen produced per flash was measured at 18°C with a rate electrode equivalent to that described by Joliot and Joliot [45]. Cells at 100 μg Chl ml^{-1} in a medium, containing 20 mM HEPES (pH 6.5), 100 mM KCl, and 5 mM MgCl$_2$, were dark-adapted for 5 min prior to each flash sequence. The short (5 μs) saturating flashes were separated by 0.5 s. The miss and double-hit parameters and the initial S_0 and S_1 apparent values were deduced using the 'sigma analysis' developed by Lavorel [46]. Oxygen evolution was also measured using a Clark-type oxygen electrode. The cells were at 10 μg Chl ml^{-1} in the growth medium.

3. Results

3.1. State transitions followed by fluorescence measurements

Fig. 1A shows a typical fluorescence trace in Synechocystis PCC 6803 cells. Dark-adapted cells presented a low F_{md} level characteristic of cyanobacteria [47–50]. Upon illumination by low intensities of blue light, exciting preferentially PS I, a maximal level of $F_{m'}$ was reached (state 1). This level was similar to F_m, obtained in the presence of light plus DCMU (Fig. 1). Then, orange illumination, preferentially exciting PS II, induced a large quenching of $F_{m'}$ (state 2). Slight changes of the $F_{0'}$ level were also observed: $F_{0'}$ was higher under blue than orange illumination or darkness (Fig. 1 and Table 1).

Fig. 1B shows the time course of the steady-state (F_s) and the maximal ($F_{m'}$) fluorescence levels of dark-adapted wild-type and mutant cells illuminated successively by blue and orange light. The different

Table 1
Fluorescence parameters during illumination with light 1 and light 2

		$F_{m'}$ (rel. units)	$F_{0'}$ (rel. units)	$F_{v'}/F_{m'}$	$F_{v'}$	NPQ
Blue	WT	1020	440	0.57	580	0
	MUT	800	410	0.49	390	0
Orange	WT	660	410	0.38	250	0.54
	MUT	652	377	0.42	275	0.22

The NPQ was quantified by means of the Stern–Volmer formulation, NPQ $= (F_m - F_{m'})/F_{m'}$, that measures the ratio of quenched to remaining fluorescence. WT: wild-type; MUT: desA$^-$/desD$^-$ mutant. $F_{m'b} = F_m$ (measured in the presence of light+DCMU).
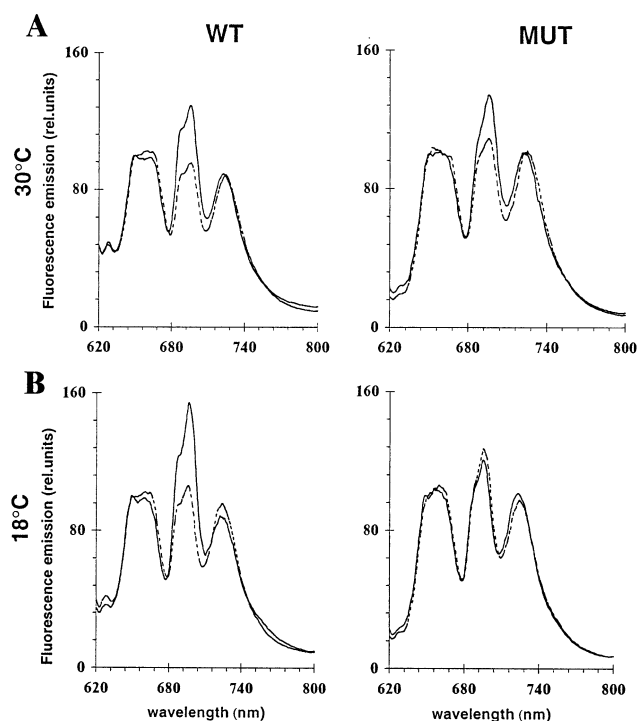
Fig. 2. Fluorescence emission spectra at 77K of dark-adapted wild-type (WT) and mutant (MUT) cells illuminated for 5 min with blue light (solid line) or orange light (dashed line) at 30°C (A) or at 18°C (B). The excitation wavelength was 605 nm. An external fluorescence probe, the fluorescein, was added to the sample. The spectra were normalized to the intensity of the fluorescein peak at 508 nm.

fluorescence parameters are described in Table 1. Comparing wild-type and mutant cells, large differences on the fluorescence parameters were observed. Under low intensities of blue illumination, the $F_{v'}$ and $F_{m'}$ levels were higher in wild-type than in mutant cells. Whereas under orange illumination, these values were similar in both strains or slightly lower in wild-type than in mutant cells. As a consequence, wild-type cells presented a larger NPQ than mutant cells (Table 1). In addition, at the intensities used, the rate of state 1 to state 2 transition was faster in wild-type than in mutant cells (Figs. 1B and 4).

Fig. 2A shows the 77K fluorescence emission spectra of dark-adapted cells illuminated for 5 min with blue or orange light at 30°C. Fluorescence emission at 720 nm originates from PS I whereas the peak at 685 nm corresponds mainly to the emission of the phycobilisome terminal emitter. Fluorescence emission from the Chl antenna CP 43 also contributes to this peak. The peak at 695 nm derives from the

Chl a emission of the PS II antenna CP 47 and the reaction center II [15,51]. Phycocyanin (at 640 nm) and allophycocyanin (at 660 nm) also contributes to the fluorescence spectrum. In both strains, the value of the F_{695}/F_{720} ratio was larger under low intensities of blue illumination than under orange illumination or darkness. Under orange illumination, a large decrease of the 685 and 695 nm peaks was observed. The amplitude of the F_{720} remained rather independent of the illumination: sometimes a slightly higher F_{720} peak was observed under light 2 illumination. The differences in the F_{695}/F_{720} ratio were smaller in mutant than in wild-type cells. In the wild-type cells, the F_{695}/F_{720} ratio varied from 1.6 under light 1 to 1.1 under light 2, while in mutant cells, it varied from 1.3 to 1.1.

Kinetics and amplitudes of fluorescence changes were determined at different temperatures. First, the temperature of the phase transition of thylakoid membranes was measured to define the state of the membrane at the different temperatures used in this study. The temperature of the phase transition of thylakoid membranes was measured by means of a native Chl a fluorescence probe. A maximum of Chl a fluorescence appeared at the temperature of phase
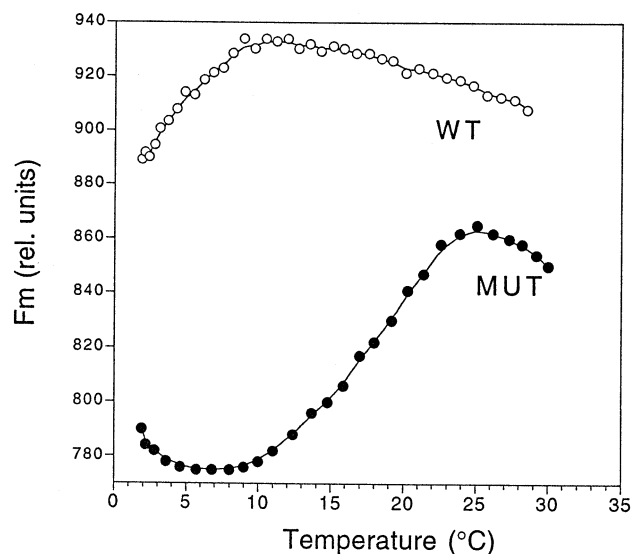

Fig. 3. Determination of the phase transition temperature of thylakoid membranes in wild-type (open circles) and mutant (closed circles) intact cells, using Chl a fluorescence. Maximal fluorescence F_m in the presence of DCMU (10 μM) under white light at 30 μmol photons m^{-2} s^{-1} was recorded and plotted against temperature from 30 to 2°C.
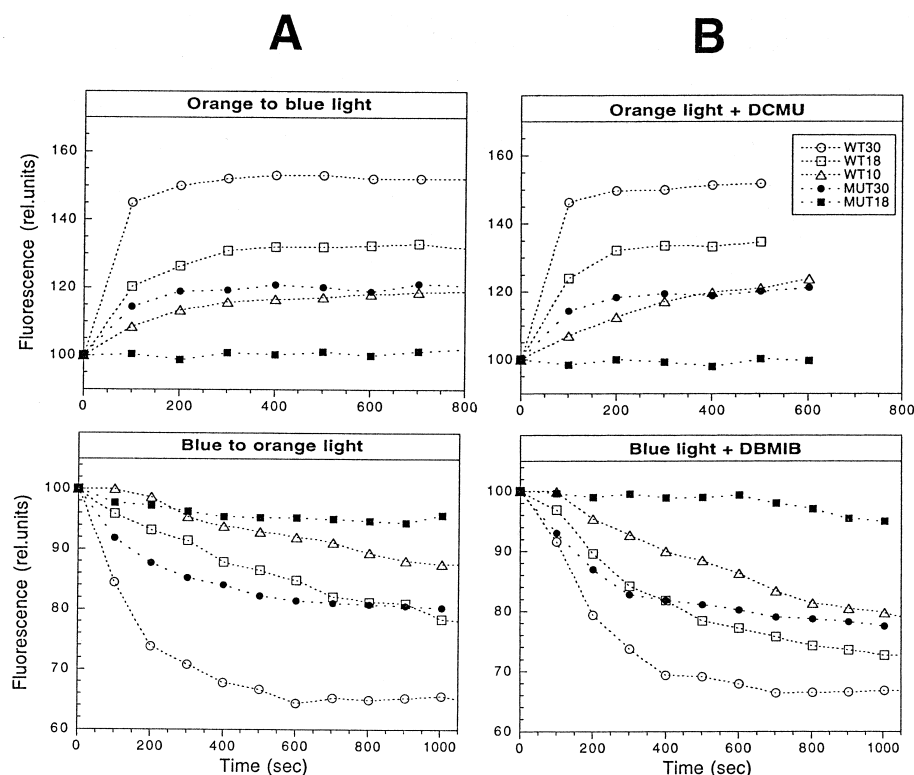
Fig. 4. Effect of temperature on state transitions in wild-type (open symbols) and mutant cells (closed symbols). (A) The upper panel shows the increase in $F_{m'}$ following the state 2 to state 1 transition from orange (O.L.) to blue (B.L.) light at 30 (circles), 18 (squares) and 10°C (triangles), while the lower panel shows the $F_{m'}$ decrease following the state 1 to state 2 transition from blue to orange light at 30, 18 and 10°C. (B) Changes in the $F_{m'}$ level in the presence of DCMU (10 μM) under orange light illumination (upper panel), or in the presence of DBMIB (10 μM) under blue light illumination (lower panel) at different temperatures (30, 18 and 10°C). Orange light was at 12 μmol photons m^{-2} s^{-1} and blue light at 20 μmol photons m^{-2} s^{-1}. Saturating pulses separated by 100 s were applied to assess $F_{m'}$ levels during the different regimes of light.

transition in curves of fluorescence versus temperature [52]. We followed the changes of F_m in the presence of DCMU under white illumination from 30 to 2°C (Fig. 3). Phase transitions of the thylakoid membranes of wild-type and the mutant cells (grown at 30°C) occurred at 12 and 25°C, respectively.

We then compared the effect of temperature on state transitions in both strains. Wild-type cells were preincubated 10 min under dark conditions and then were successively illuminated by orange light for 15 min and then by blue light (state 2 to state 1 transition) or, by blue light (15 min) followed by orange light (state 1 to state 2 transition) at 30, 18, 10 and 2°C. In the later case, the lowering of temperature was carried out under blue illumination. Differences in the amplitude and in the kinetics of state transitions were observed (Fig. 4A). The rates of $F_{m'}$ increase and decrease were slowed down by

lowering the temperature. The $t_{1/2}$ of the transition to state 2, increased from 120 s at 30°C to 600 s at 10°C. The complete transition from state 2 to state 1 occurred in 150 s at 30°C ($t_{1/2} = 50$ s) and in 700 s at 10°C ($t_{1/2} = 160$ s) (Fig. 4A). In addition, when the temperature decreased from 30 to 18 and 10°C, the $F_{m'b}$ level progressively decreased and the $F_{m'0}$ level increased (data not shown) resulting in a smaller amplitude of state transitions. The q_N decreased with the temperature, from 0.58 at 30°C to 0.23 at 10°C. At 2°C, the thylakoid membranes were in the solid state and there were no state transitions. The cells remained in the low fluorescence state characteristic of dark conditions (data not shown).

Artificial reduction or oxidation of the plastoquinone pool (PQ pool) also induces changes on $F_{m'}$ levels similar to that observed during state transitions generated by preferential illumination of one of the
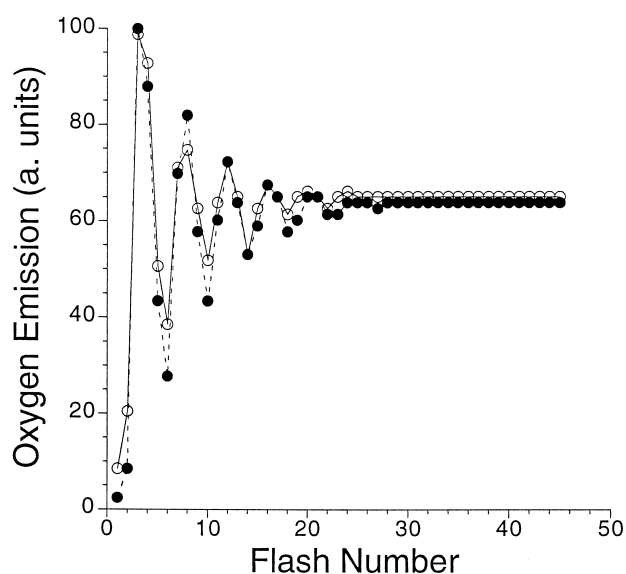
Fig. 5. Oxygen yield (in relative units) per flash during a series of short (5 μs) saturating flashes spaced by 0.5 s in wild-type (open circles) and mutant (closed circles) cells. The sequence was measured at 18°C. The values of the oxygen sequence parameters were computed by the matrix analysis from recorded oxygen sequences on samples dark-adapted for 5 min. S_0 (concentration in the dark-adapted state): 34% (WT), 36% (MUT); S_1 (concentration in the dark-adapted state): 66% (WT), 63% (MUT); α (miss parameter): 0.18 (WT), 0.16 (MUT).

photosystems [50,53]. DCMU inhibits the reduction of the PQ pool (via the PS II) by binding to the Q_B site in the D1 protein of the reaction center II. 2,5-Dibromo-3-methyl-6-isopropyl-benzoquinone (DBMIB) inhibits the reoxidation of the PQ pool. It binds to the Qo site of the cytochrome b_6f complex and blocks the transfer of electrons from the PQ pool to the cytochrome b_6f complex. Fig. 4B shows changes on $F_{m'}$ induced by addition of DCMU or DBMIB at different temperatures. DCMU (10 μM) was added 5 min after the beginning of the orange illumination. DCMU addition immediately induced the closure of all PS II centers, then a transition to a high fluorescence state (similar to state 1) was observed. The rate of this transition was slowed down by lowering the temperature ($t_{1/2}$ at 30°C = 50 s; $t_{1/2}$ at 10°C = 200 s) (Fig. 4B). DBMIB (10 μM) was added to the cells 5 min after the beginning of the blue illumination. Upon DBMIB addition, a transient increase of the F_s level was observed indicating the closure of some PS II centers as consequence of the reduction of the PQ pool. Then, large F_s and $F_{m'}$

quenchings were induced. The $t_{1/2}$ of fluorescence quenching increased from 150 to 600 s by lowering the temperature from 30 to 10°C (Fig. 4B). At 2°C, both transitions were completely inhibited (data not shown).

In mutant cells, the kinetics of state transitions were determined at two temperatures: 30 and 18°C, respectively corresponding to the liquid crystalline and solid state of the thylakoids (Fig. 4). Even at 30°C the kinetics of state transition were slower in the mutant than in the wild-type (Fig. 4). At 18°C the state transitions were completely blocked (Figs. 2 and 4). As already mentioned, the amplitude of the state transitions was smaller in the mutant due to a lower $F_{m'}$ value under blue light.

3.2. PS II activity determined by oxygen sequences

The rate of diffusion of the PQ molecules and the rates of reduction and reoxidation of the PQ pool may depend on the temperature. These changes could influence the rate of state transitions. A way to detect changes in the rate of PQ reduction and/or oxidation is to measure the amount of oxygen produced per flash in dark-adapted cells during a train of saturating flashes. Activation of dark-adapted samples by a train of saturating short flashes produces oxygen with a yield per flash that oscillates with a periodicity of four [54,55]. After several flashes, the amplitude of the oscillations diminishes until a constant oxygen yield per flash. This damping is due to double turnovers (double hits) occurring during the flashes or to misses. When the reoxidation of the PQ pool is slowed down, Q_A^- is present in a larger quantity of centers before the flash, inducing the increase of misses and the decrease of the Ym level (constant oxygen level reached after a series of flashes). To elucidate whether the reoxidation of the PQ pool was slower in the mutant than in the wild-type at 18°C, we measured the oxygen produced per flash in each strain.

Fig. 5 shows that the oscillations of oxygen produced per flash in dark-adapted wild-type and mutant cells at 18°C. Both displayed a maximum amount on the third flash and were similar (Fig. 5). The dark concentration of S_0 and S_1 and the miss and double hit parameters were similar in wild-type and mutant cells (legend of Fig. 5). The Ym level did
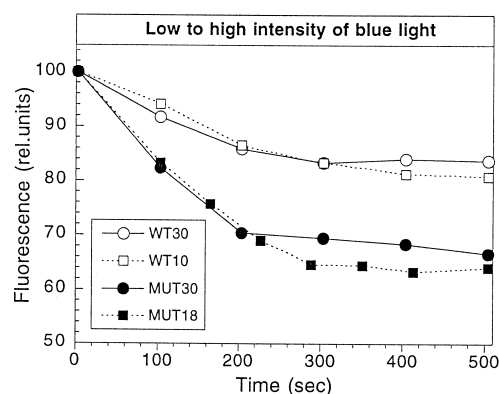
Fig. 6. Changes in the $F_{m'}$ level in wild-type (open symbols) and mutant cells (closed symbols), at 30 (circles) and 18°C (squares), induced by blue light at 440 μmol photons m^{-2} s^{-1}. Cells adapted for 5 min to low intensities of blue light (20 μmol photons m^{-2} s^{-1}) were exposed to 400 μmol photons m^{-2} s^{-1} blue light. Saturating pulses were applied to assess $F_{m'}$.

not decrease in mutant or in wild-type cells indicating no limitation of oxygen evolution by PQ reoxidation (Fig. 5). Oxygen evolution measurements carried out with a Clark-type electrode showed similar activities in wild-type and mutant cells (data not shown and [41]). These results suggested that the diffusion of the PQ pool was not largely affected by the unsaturation of thylakoid membrane lipids as already proposed by Gombos et al. [56].

3.3. NPQ induced by strong light

In cyanobacteria cells, light intensities at least two or three fold higher than that of growth conditions induce NPQ [40]. We have further characterized this type of quenching and its temperature dependency by using two intensities of strong blue light. We used blue light, exciting preferentially PS I, in order to avoid over-reduction of PS II leading to photo-inhibition. At 400 μmol photons m^{-2} s^{-1}, only 15% of PS II centers were closed and oxygen evolving activity was only 30% of the maximal oxygen activity measured using DCBQ (2,6 dichloro-benzoquinone) as an external electron acceptor. At 1000 μmol photons m^{-2} s^{-1}, about 50% of PS II centers were closed and 70% of maximal oxygen evolution was observed. Upon transfer of cells adapted to low intensities of blue light (20 μmol photons m^{-2} s^{-1}) to higher blue light intensities (440 and 1000 μmol photons m^{-2} s^{-1}) a fluorescence quenching was developed in both strains (Figs. 6, 7 and Table 2). $F_{m'}$ and $F_{0'}$ were both quenched. The NPQ generated was larger in mutant cells than in wild-type cells. It is important to point out that the fluorescence quenching was induced even when only few PS II centers were closed and oxygen evolving activity was far from saturation. Under these conditions, the PQ pool might be largely oxidized due to the high PS I activity observed under blue illumination.

The quenching induced by high intensities of blue light was observed also at lower temperatures (Fig. 6). In mutant cells, even at 18°C a large fluorescence quenching was detected (Fig. 6). Although the amount of closed PS II centers increased by lowering the temperature, the amplitude of the quenching was similar at both tested temperatures in wild-type and mutant cells (Table 2 and Fig. 6). The fact that the blue light-induced quenching occurred also at low temperatures even when the thylakoidal membranes were in the solid state suggested that this quenching was produced by another mechanism than that involved in state transition.

In *Synechocystis* cells, high intensities of white

Table 2
q_P and NPQ induced by high intensities of blue light

		q_P	NPQ
Blue light, 30°C (440 μmol photons m^{-2} s^{-1})	WT	0.85 ± 0.03	0.22 ± 0.03
	MUT	0.86 ± 0.02	0.53 ± 0.02
Blue light, 30°C (1000 μmol photons m^{-2} s^{-1})	WT	0.53 ± 0.02	0.35 ± 0.02
	MUT	0.55 ± 0.03	0.70 ± 0.02
Blue light, 10°C (440 μmol photons m^{-2} s^{-1})	WT	0.45 ± 0.02	0.21 ± 0.03
Blue light, 18°C (440 μmol photons m^{-2} s^{-1})	MUT	0.63 ± 0.02	0.54 ± 0.02

$q_P = (F_{m'} - F_s)/(F_{m'} - F_{0'})$, ($q_P = 1$, all PS II centers are open). The NPQ was quantified by means of the Stern–Volmer formulation, NPQ $= (F_m - F_{m'})/F_{m'}$, that measures the ratio of quenched to remaining fluorescence. WT: wild-type; MUT: desA$^-$/desD$^-$ mutant.
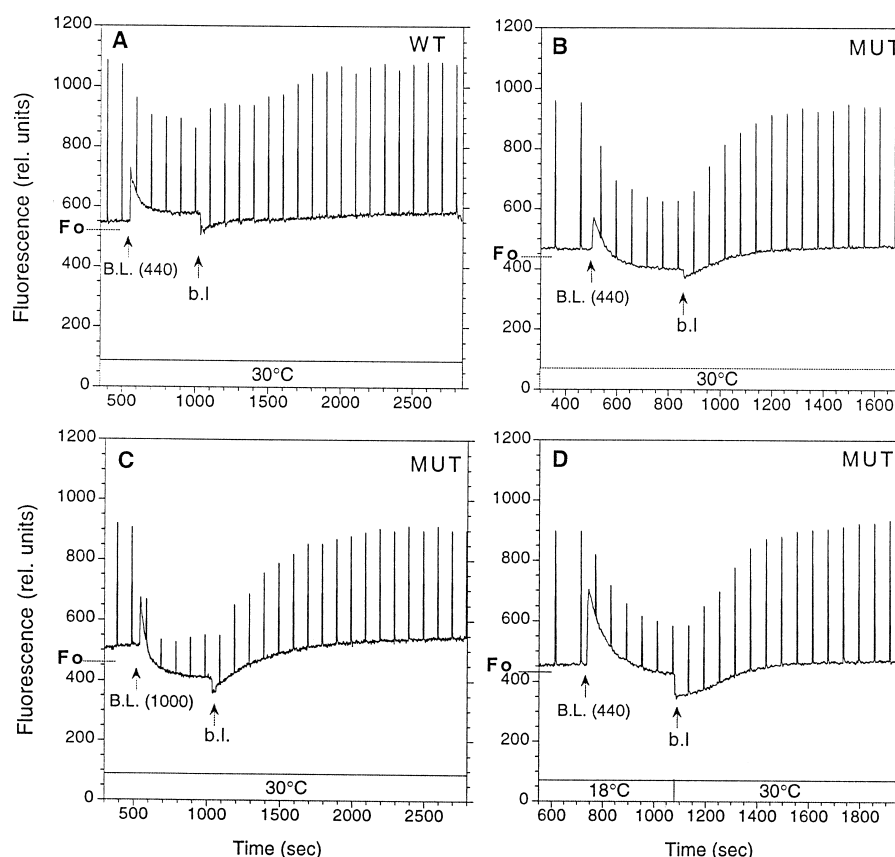
Fig. 7. Effect of lincomycin on the recovery process. Changes in the fluorescence yield in wild-type (A) and mutant cells (B, C, D) induced by blue light at 440 µmol photons m^{-2} s^{-1} (B.L. 440) (A, B, D) or 1000 µmol photons m^{-2} s^{-1} (B.L. 1000) (C) in the presence of lincomycin at 30°C (A, B, C) or 18°C (D). In all cases, recovery under blue light 20 µmol photons m^{-2} s^{-1} (B.L.) was carried out at 30°C. Lincomycin (300 µg ml^{-1}) was added in darkness, 5 min before illumination.

light induce a large fluorescence quenching always followed by the inhibition of PS II activity and D1 degradation (photoinhibition). Recovery of this type of fluorescence quenching requires active protein synthesis. In order to test whether the fluorescence quenching induced by blue light could be partially due to D1 damage, we illuminated the cells in the absence or in the presence of lincomycin, an inhibitor of protein synthesis. Fluorescence quenching was induced by two intensities of blue light (440 and 1000 µmol photons m^{-2} s^{-1}) at 18 or 30°C. When quenched cells were transferred again to low intensities of blue light (20 µmol photons m^{-2} s^{-1}) they recovered their maximal level of $F_{m'}$ even in the presence of lincomycin (Fig. 7). The recovery kinetics were similar in the presence and absence of lincomycin (data not shown). In contrast to the rate of quenching formation, recovery was slightly slower

at lower temperatures (data not shown). In mutant cells, maximal $F_{m'}$ was recovered after 10 min at 30°C while at 18°C, only 80% of $F_{m'}$ was recovered in the same time. Nevertheless, the fluorescence quenching generated at 18°C was completely dissipated when cells were shifted to 30°C under blue light at 20 µmol photons m^{-2} s^{-1} even in the presence of lincomycin (Fig. 7). These results indicated that the fluorescence quenching induced by high intensities of blue light at 30 and 18°C was not related to D1 damage.

4. Discussion

Our results about the characterization of PS II fluorescence changes induced in the wild-type and in a genetically engineered strain of Synechocystis

PCC 6803 lacking polyunsaturated fatty acids strongly support the idea that two different mechanisms are involved in state transition and high light induced quenching: one, dependent on the fluidity of the membrane and the other, independent.

4.1. State transitions

The structural changes associated with state transitions in cyanobacteria are still a matter of discussion. Several models were proposed: (1) Migration of the phycobilisome from the PS II to the PS I inducing a change of the size antenna of both photosystems [23,48]. (2) Migration and/or oligomerization/monomerization of the photosystems inducing changes in energy transfer from the phycobilisomes to each photosystem [25,57] or in spillover [17,24].

The results described in this article are more in favor for the photosystems being the mobile elements in state transitions. We showed that in *Synechocystis* PCC 6803 wild-type cells, the fluorescence emission changes, induced by a selective illumination of one of the two photosystems, were dependent on the temperature: the amplitude and the rate of state transitions decreased upon lowering the temperature. Moreover, state transitions were completely inhibited at 2°C, at which temperature the thylakoid membranes are in the solid crystalline state. Fork et al. [47] and Williams et al. [58] have already observed that the fluorescence changes associated with dark-light acclimation sharply decreased by lowering the temperature in other strains of cyanobacteria. The decrease in the amplitude of the fluorescence changes can be interpreted by assuming that the rigidification of the membrane not only slows down the movement but also decreases the number of complexes involved in state transitions.

In *desD⁻/desA⁻* mutant cells of *Synechocystis* PCC 6803, in which the membranes are more rigid due to the absence of di- and tri-unsaturated fatty acids, state transitions were inhibited at higher temperatures than in wild-type cells. In these mutant cells, state transitions were already modified at 30 and 25°C; the amplitude was very small and the rate was very low. At 18°C, state transitions were completely inhibited in mutant cells while in wild-type cells large fluorescence emission changes still occurred at a high rate. The fluorescence emission changes induced by illumination of cells in the presence of electron transport inhibitors, DCMU and DBMIB, presented the temperature and membrane fluidity dependence similar to those induced by specific light illumination in both strains. We can conclude that similar mechanisms are involved in the fluorescence changes induced by different qualities of light or by the redox state of the PQ pool (and/or PS I activity). Our results can be interpreted as an indicator that the fluidity of the thylakoid membrane plays a role in state transitions.

Comparison of the oscillations of oxygen produced per flash during illumination by a series of saturating white flashes, in mutant and wild-type cells, indicated that no serious limitation of PQ re-oxidation occurred at 18°C in the mutant. These results suggested that the inhibition of state transitions at 18°C in mutant cells was not related to large changes in the rate of photosynthetic electron transport. We propose, as a working hypothesis, that in mutant cells, at 18°C, the inhibition of state transitions is due to the impediment of the movement of the pigment-protein complexes in the bulk of lipids of the membrane. It has been shown that in higher plants, where the diffusion of the membrane LCHII is involved in state transition mechanism, the rigidification of the thylakoid membrane by addition of cholesterol inhibits state transitions and PS II–PS I segregation [59]. Results of other laboratories have also suggested that in cyanobacteria the mobile elements are the photosystems. State transitions are accompanied by ultrastructural changes of the thylakoid membranes: in state 1, a larger quantity of PS II complexes are arranged in rows compared to state 2 (40% versus 20%) [16,24]. A mutant containing only monomeric PS I complexes is capable of performing state transitions more rapidly than the wild-type containing trimeric forms suggesting that PS I complexes rather than phycobilisomes might move [25]. Variations of fluorescence emissions from PS II and PS I induced by light 1 or light 2 seems to occur also in phycobilisome-less mutants [24,60].

Recently, Rögner and co-workers [57] proposed a new model for state transitions that involves different oligomeric forms of PS II and PS I in addition to a reorganization of the photosystems in the thylakoids. In this model, state 1 has a dimeric PS II and a monomeric PS I, and state 2 has a monomeric PS

II and a trimeric PS I. Preliminary experiments with thylakoids of a thermophilic cyanobacterium have showed that monomerization of PS I by high salt treatment occurs only at temperatures higher than 35°C at which the membrane is in the liquid phase [57]. The increase in the rigidity of the thylakoid membranes certainly also hinders the oligomerization of the photosystems and in this way could inhibit state transitions.

We cannot exclude the hypothesis that the movement of phycobilisomes may also be slowed down by the rigidification of the membrane. The phycobilisomes are anchored to the PS II and this interaction may be influenced by the state of the membrane. However, our results support the hypothesis that the movement of at least one membrane pigment-protein complex and/or changes in the oligomerization state of one or more complexes are involved in the mechanism of state transitions.

4.2. PS II fluorescence quenching induced by high light intensities

In higher plants and green algae, state transitions are the only form of quenching observed under weak illumination. Under strong illumination, the ΔpH-dependent quenching is predominant. In red algae illumination with weak light 2 or strong white light a large ΔpH-dependent quenching is induced [38,39]. Near-saturating and saturating intensities of white light also induce PS II fluorescence quenching in cyanobacteria. Since addition of uncouplers did not suppress this quenching [40], Campbell et al. suggested that the quenching induced by high light intensities is related to state transitions and/or photoinhibition. However, a recent work, describing a Synechocystis PCC 6803 mutant unable to perform state transitions, showed that this mechanism is physiologically important only at very low light intensities [61].

Data about strong light effect presented in this work point towards a mechanism different from that involved in photoinhibition or state transitions. During photoinhibition, the D1 protein of the reaction center II is damaged and has to be replaced by a newly synthesized protein in order to recover the lost variable fluorescence and oxygen evolution activity (for reviews see [6,7]). Inhibition of protein synthesis

avoids the recovery process. The fact that the quenching induced by strong blue light (at any temperature) was suppressed when wild-type and mutant cells were transferred to low blue light intensities, even in the presence of an inhibitor of protein synthesis, indicates that this quenching is not associated with an irreversible damage of D1 and photoinhibition.

Excitation pressure on PS II or reduction of the PQ pool does not seem to be the trigger for the quenching induced by high intensities of blue light as it appears to be the case in transition to state 2 or photoinhibition. The intensities of blue light that induce this quenching are not saturating for the oxygen evolving activity. It can occur when only a small number of closed PS II is accumulated. In cyanobacteria, PS II centers remain open under a large range of high light intensities due to their high PS I/PS II ratio. Under blue light, a high PS I activity relative to that of PS II increases this phenomenon. As a consequence, the PQ pool is largely oxidized.

The most significant result was that in Synechocystis cells, the quenching induced by strong blue or white light was not dependent on the temperature or on the membrane state. At a determined light intensity, lowering the temperature provokes the closure of more reaction centers and the reduction of the electron transport chain without significantly changing the NPQ. In $desA^-/desD^-$ mutant cells a large quenching was induced at 30°C as well as at 18°C. The amplitude of the quenching was larger in the mutant cells than in the wild-type cells. Thus, the mechanism involved in the formation of the blue-light induced quenching seems to be unrelated to the movement of membrane complexes.

One can consider that as in higher plants, in cyanobacteria a partial thermal dissipation of the absorbed energy might exist in the phycobilisome that can serve as a protective mechanism from strong light. Not only the maximal fluorescence level, $F_{m'}$, was strongly quenched by high light intensities but also a large quenching of the minimal fluorescence level, $F_{0'}$, was observed. The simultaneous quenching of $F_{m'}$ and $F_{0'}$ is generally interpreted as an antenna-based quenching mechanism. Experiments are in progress to further characterize the high-light induced quenching. Preliminary results suggest that indeed, high light intensities induced phycobilisome

fluorescence quenching accompanied by a decrease of energy transfer from the phycobilisome to both PS II and PS I.

In conclusion, our results clearly demonstrated that in the cyanobacterium *Synechocystis* PCC 6803, the non-photochemical PS II quenching induced by strong blue light is unrelated to state transition or photoinhibition suggesting that in cyanobacteria a third type of NPQ may exist that remains to be further characterized.

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