The Slow S to M Fluorescence Rise is Missing in the RpaC Mutant of *Synechocystis sp.* (PCC 6803)

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Abstract: Transfer of phycobilisome-containing cyanobacteria from darkness to continuous light results in a typical chlorophyll a fluorescence induction that follows a pattern labeled as OJIPSMT. This pattern of fluorescence induction (FI) reflects changes in both photochemical and non-photochemical processes. We have focused on the slow S to M fluorescence rise that is dominant in cyanobacteria. We clearly observe the S-to-M fluorescence rise in the wild type (WT*)* cells of *Synechocystis sp.* (PCC 6803) in the presence of 1, 1'-dimethyl-3(3'4'-dichloro)-phenylurea (DCMU). This rise is fully suppressed by hyperosmotic glycine betaine that prevents the mobility of extramembrane phycobilisomes. The contribution of the State 2-to-State 1 transition in the S-to-M rise of WT cells was proven by changes in the 77 K emission spectra: the spectra at point O of FI (*i.e.*, in State 2, with lower ratio of F685/F726) were characteristically different from those at point M (*i.e.*, in State 1, with higher ratio of F685/F726). The S-M rise was totally missing in RpaC– mutant of *Synechocystis sp.* (PCC 6803) that is locked in the high-fluorescence State 1 and thus is unable to do state transitions. Moreover, RpaC- mutant showed quenching of phycobilin fluorescence during the S-M rise period. Taken together, these data suggest that the State 2 to State 1 transition is the dominant cause of the S to M fluorescence rise in cyanobacteria.

Keywords: PH–related fluorescence quenching; Cyanobacteria; Fluorescence induction; S to M fluorescence rise; State changes

Introduction

Kinetics of chlorophyll a fluorescence in photosynthetic organisms follows a typical pattern after irradiation: O-J-I-P-S-M-T transition (O—initial, J and I—inflections, P—peak, S—semi-steady state, M—maximum, T—the terminal steady state fluorescence). The fast part of the fluorescence induction curve (during the first second of actinic irradiance) is dominant in higher plants but rather shallow in cyanobacteria. On the contrary, the slower fluorescence increase (in tens of seconds) from the S-plateau to the M peak (Papageorgiou and Govindjee 1968) is dominant in cyanobacteria (Papageorgiou *et al*., 2007). Since cyanobacteria tend to stay in the low fluorescence State 2 during dark and are transformed into the high fluorescent State 1 during irradiation (Stamatakis *et al.*, 2007), the S to M fluorescence rise has been assigned mostly to an increase in PBS (phycobilisome) → PS II excitation transfer (Papageorgiou *et al*., 2007). The higher PBS → PS II excitation transfer accompanied by stimulation of S to M rise can be also seen in a case of inhibition in PBS → PS I excitations transfer (Papageorgiou *et al.*, 2007; Stamatakis *et al.*, 2007). Such a redistribution in energy transfer from PBS is possibly allowed either by phycobilisomes mobility (Joshua and Mullineaux, 2004) or by some other energy re-distribution mechanism (McConnell *et al*., 2002). As the S to M rise can be observed also in the presence of DCMU

(Papageorgiou and Govindjee, 1968), it rules out an involvement of electron transport between Q_B to PQ-pool (Tsimilli-Michael *et al.*, 2009). This phenomenology suggests that the S to M rise reflects regulatory distribution of PBS excitation to PSII and PSI. Here, we have further investigated the validity of this hypothesis by comparing the FI patterns of chlorophyll *a* fluorescence measured with wild type (WT) and with state-transition mutant (RpaC–) of *Synechocystis sp.* PCC6803 cells (Emlyn-Jones *et al*., 1999). Thus, we demonstrate here that the S-to-M fluorescence rise is related to State 2 to State 1 transition.

Materials and Methods

Synechococystis sp. (PCC 6803) cells were cultivated in BG 11 medium in an orbital shaking incubator at 28 °C at continual irradiance of 14 μmol (photons) m^{-2} s^{-1}. Fluorescence induction at Chl a fluorescence maximum was measured between 690–710 nm with a double modulated fluorimeter FL-100 (PSI, Czech rep.). The fluorescence spectra during FI was detected by Spectrally Resolved Fluorescence Induction (SRFI) method (see *e.g.* Kaňa *et al.*, 2009) at actinic irradiation (orange light 590 nm, ~300 μmol (photons) m^{-2} s^{-1}) and during saturating flash (590 nm, 1,500 μmol (photons) $m^{-2}s^{-1}$). The spectra were detected by a diode array spectrophotometer SM-9000 every 100 ms (PSI, Brno, Czech Republic). The low temperature (77 K) fluorescence emission spectra were detected at liquid nitrogen by spectrophotometer SM-9000 (PSI, Brno, Czech Republic).

Results and Discussion

We have observed a typical kinetic fluorescence pattern during dark-light transition in wild type (WT) *Synechocystis sp.* (PCC 6803) cells that showed both the fast OJIP transient and the slower S-M transition (see Fig. 1A). However, in *Synechocystis sp.*, the S-M rise was less pronounced (Fig. 1) than in *Synechococcus sp.* (Kaňa *et al.*, 2009). In fact, it was clearly visible with presence of DCMU (see the DCMU curve in Fig. 1A) in agreement with the previous results (Papageorgiou and Govindjee, 1968). As the S to M rise is clearly visible in the presence of DCMU (Fig. 1), it must be somehow controlled by the redox state of the PQ pool. It may mean that when PSII reaction center is closed by DCMU, PQ pool become mostly oxidized upon illumination and this may result in PBS redistribution in favor of PBS → PS II excitation transfer – and, thus, we see strong S-M fluorescence rise (Fig. 1).

We have further studied properties of the S-M rise in *Synechocystis sp.* and found that it is abolished by hyperosmotic glycine betaine (Fig. 1A). It is known that hyper-osmotic conditions in general block the SM rise in PBS-containing cyanobacteria reversibly, without blocking the OJIPS phase (Stamatakis *et al.*, 2007). It seems that glycine betaine blocks PBS redistribution from PS I to PS II during irradiation. This result can be either due to blocking of phycobilisome diffusion mobility (Li *et al.*, 2004) or due to an inhibition of some other mechanism affecting PBS → photosystem energy transfer. Such a regulatory re-distribution of PBS excitation is known as State 2-to-State 1 transition (see *e.g.* McConnell *et al.*, 2001; Mullineaux and Emlyn-Jones, 2005).

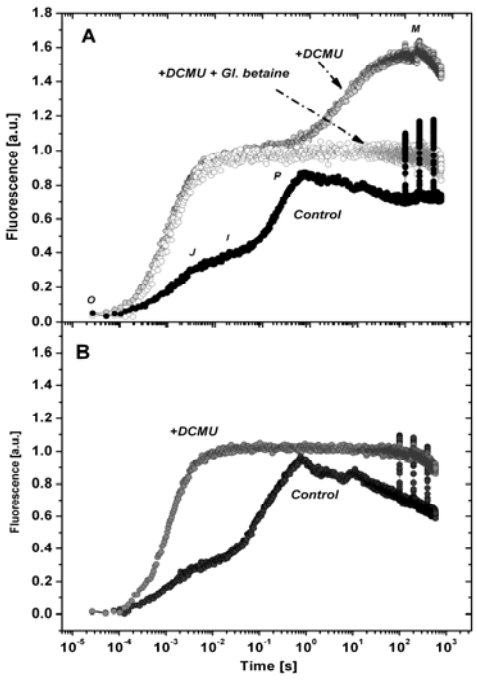

Fig. 1 Time course of chlorophyll a fluorescence induction (FI) of wild type cyanobacterium *Synechocystis sp.* (panel A) and its mutant without RpaC protein (panel B). Cells were dark adapted and exposed to orange excitation light (590 nm, 300 μmol (photons) m^{-2} s^{-1}). All curves are from the same sample before and after addition of inhibitors. First, the control curve was obtained ("Control") that was followed by the addition of 10 μM DCMU ("DCMU") and finally 520 mmol glycine betaine was added ("Gl. betaine"). Data are normalized to the maximal fluorescence before measurements. Characteristic peaks during fluorescence transient (OJIPM) are marked.

The connection between S-M fluorescence rise and State 2 to State 1 transition was shown by measurements with state-transition mutant (RpaC–) of *Synechocystis sp.* (Joshua and Mullineaux, 2005) that is unable to carry out state transition changes. Indeed, in the case of RpaC– mutant, there is no fluorescence rise even after addition of DCMU (Fig. 1B). This confirms that state transition changes are crucial for S-M fluorescence rise in *Synechocystis sp.*

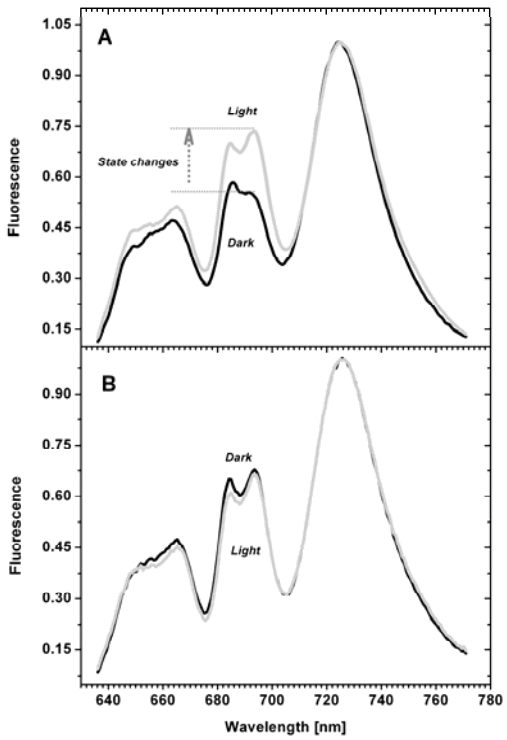

Fig. 2 Low temperature (77 K) fluorescence emission spectra measured with WT (panel A) and RpaC- mutant (panel B) of *Synechocystis sp*. Data were obtained after 20 min of dark, see "Dark" curves or after 200s long irradiation with orange light (590 nm, 300 μmol (photons) m^{-2} s^{-1}) - see "Light" curves. Fluorescence was exited at 530 nm. DCMU (10 μM) was added before measurements. Fluorescence increase due to State 2 to State 1 transition on light is marked. Data are normalized to fluorescence at 726 nm.

We have further explored the connection between state changes and the S-M fluorescence rise by low temperature (77 K) fluorescence spectroscopy. It is well known that PBS redistribution from PSI to PSII causes relative increase in PSII fluorescence (between 685–695 nm) in comparison to PSI fluorescence (with maximum at 726 nm). Indeed, such a stimulation of PSII fluorescence at 685–695 nm was visible after irradiation of WT cells of *Synechocystis sp.* treated with DCMU (Fig. 2A). On the contrary, there was no increase in fluorescence of PSII after irradiation in RpaC- mutant (Fig. 2B). Since the RpaC– mutant cells are locked in the high-fluorescence State 1 any redistribution of PBS is blocked during irradiation and thus there is no S-M fluorescence rise in RpaC– mutant of *Synechocystis sp*. (Fig. 1B). All the above mentioned data, taken together, suggest that the State 2 to State 1 transition is the dominant cause of the S to M fluorescence rise in cyanobacteria.

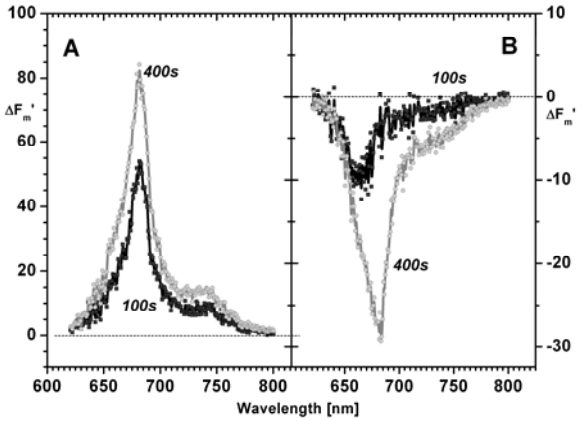

Fig. 3 Typical difference fluorescence emission spectra measured with saturated light pulses (spectra of maximal fluorescence - F_m). The ΔF_m' spectra represent the difference, F_m'- F_m, where F_m is maximal fluorescence measured in the dark and F_m' is maximal fluorescence measured after a particular time of irradiation (black line – 100 s; grey line – 400 s). Panel A represents difference fluorescence spectra of WT, and the panel B shows data obtained with RpaC- mutant. The curves shown were obtained from raw data measured by Spectrally Resolved Fluorescence Induction – SRFI method (see Materials, Methods, Kaňa *et al*., 2009).

We have also briefly explored the effect of high irradiance during the period of S-M rise in the RpaC- mutant that is incapable of PBS redistribution between photosystems, and is permanently locked in State 1 (Fig. 2B). We have measured room temperature spectra of maximal fluorescence for dark adapted sample at saturating flash (F_m spectra) and compared them with the spectra measured after 100 s and 400 s of light – the $\Delta Fm' = F_m - F_m'$ difference spectra are presented in Fig. 3. We observe (Fig. 3 A) that the irradiation of WT cells caused increases in maximal fluorescence that reflect State 2 to State 1 transition as already shown in Fig. 1 and Fig. 2. However, in the case of RpaC- mutant of *Synechocystis sp.* there decreases in maximal fluorescence after irradiation (see difference spectra ΔF_m' in Fig. 3B) due to some non-photochemical

processes because ΔF_m' was measured at saturating flashes, when reaction centers are closed. Thus, we can conclude that the inability of state transition in RpaC- mutant results in non-photochemical quenching of fluorescence.

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