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Fluorescence induction in the phycobilisome-containing cyanobacterium *Synechococcus* sp PCC 7942: Analysis of the slow fluorescence transient

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Abstract

At room temperature, the chlorophyll (Chl) *a* fluorescence induction (FI) kinetics of plants, algae and cyanobacteria go through two maxima, P at ~0.2–1 and M at ~100–500 s, with a minimum S at ~2–10 s in between. Thus, the whole FI kinetic pattern comprises a fast OPS transient (with O denoting *origin*) and a slower SMT transient (with T denoting *terminal state*). Here, we examined the phenomenology and the etiology of the SMT transient of the phycobilisome (PBS)-containing cyanobacterium *Synechococcus* sp PCC 7942 by modifying PBS→Photosystem (PS) II excitation transfer indirectly, either by blocking or by maximizing the PBS→PS I excitation transfer. Blocking the PBS→PS I excitation transfer route with N-ethyl-maleimide [NEM; A. N. Glazer, Y. Gindt, C. F. Chan, and K.Sauer, Photosynth. Research 40 (1994) 167–173] increases both the PBS excitation share of PS II and Chl *a* fluorescence. Maximizing it, on the other hand, by suspending cyanobactrial cells in hyper-osmotic media [G. C. Papageorgiou, A. Alygizaki-Zorba, Biochim. Biophys. Acta 1335 (1997) 1–4] diminishes both the PBS excitation share of PS II and Chl *a* fluorescence. Here, we show for the first time that, in either case, the slow SMT transient of FI disappears and is replaced by continuous P→T fluorescence decay, reminiscent of the typical P→T fluorescence decay of higher plants and algae. A similar P→T decay was also displayed by DCMU-treated *Synechococcus* cells at 2 °C. To interpret this phenomenology, we assume that after dark adaptation cyanobacteria exist in a low fluorescence state (state 2) and transit to a high fluorescence state (state 1) when, upon light acclimation, PS I is forced to run faster than PS II. In these organisms, a state 2→1 fluorescence increase plus electron transport-dependent dequenching processes dominate the SM rise and maximal fluorescence output is at M which lies above the P maximum of the fast FI transient. In contrast, dark-adapted plants and algae exist in state 1 and upon illumination they display an extended P→T decay that sometimes is interrupted by a shallow SMT transient, with M below P. This decay is dominated by a state 1→2 fluorescence lowering, as well as by electron transport-dependent quenching processes. When the regulation of the PBS→PS I electronic excitation transfer is eliminated (as for example in hyper-osmotic suspensions, after NEM treatment and at low temperature), the FI pattern of *Synechococcus* becomes plant-like.

Keywords: Chlorophyll fluorescence; Cyanobacteria; Fast and slow fluorescence induction; State transitions

1. Introduction

The dynamics of electronic excitation present at any moment in the photosynthetic pigments is most dramatically expressed by the fluorescence output of chlorophyll (Chl *a*) *in vivo*, whose kinetics in the μs-to-min time range are known as *fluorescence induction* (FI; also as *the Kautsky transient*, after Kautsky and Hirsch [1]). At physiological conditions, FI consists of an initial transient (OPS, μs to s) followed by a slower transient (SMT, s to min; see reviews by Govindjee and Papageorgiou. [2],

Abbreviations: APC, allophycocyanin; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; de-qN, nonphotochemical fluorescence dequenching; de-qP, photochemical fluorescence dequenching; de-qT, fluorescence increase due to state 2→1 transition; FI, chlorophyll *a* fluorescence induction; HEPES, N-2-(2-hydroxyethyl)-N′-ethanesulfonic acid; NEM, *N*-ethylmaleimide; PBS, phycobilisome; PS I(II), photosystem I(II); PSET, photosynthetic electron transport; PQ, plastoquinone; qN, nonphotochemical quenching; qP, photochemical quenching; qT, fluorescence lowering due to state 1→2 transition

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Mohanty and Govindjee [3], Papageorgiou [4], and Govindjee [5,6].

These kinetics have been studied extensively in leaves and subcellular preparations thereof, and in algae, but less so in cyanobacteria, particularly with regard to the slow induction transient. Characteristically, in a recently published volume on Chl *a* fluorescence [7] the slow FI is briefly mentioned only in 2 chapters out of 31. In the present paper we focus on the most plant-unlike slow induction transient (SMT) of this oxygenic prokaryote.

Three classes of processes control the output of Chl *a* fluorescence *in vivo* in the μs-to-min time window: (i) photochemical quenching (qP) and dequenching (de-qP) processes that are related to the primary charge separation. (ii) Nonphotochemical quenching (qN)/de-quenching (de-qN) processes that are indirectly related to and regulated by the photosynthetic electron transport (PSET). (iii) Fluorescence lowering (qT) or fluorescence increase (de-qT) due to the state 1→2 and state 2→1 transitions [8,9]; in a strict sense, they are not true quenching/dequenching processes, since fluorescence lifetimes are not affected. We shall adhere to the qT/de-qT notation only for simplicity.

In plant leaves and algae, the full FI patterns have been rationalized in terms of the interplay of the qP, qN and qT processes. Thus, the O(JI)P fluorescence rise (where J and I denote inflections) was found to be dominated by qP/de-qP processes [10–12], with some nonphotochemical contribution by the plastoquinone (PQ)-pool [13,14], the PS decay to be dominated equally by qP and qE (mostly acidification) of the lumen [15–17], the SM rise by de-qP and de-qN, with de-qP being predominant [16,18] and the MT decay primarily by qN [16] with minor contributions by qP [19,20]. State transitions have been implicated in the P→T decay of higher plants [21,22]. Lumen acidity signals the initiation of xanthophyll cycle-dependent quenching only in the eukaryotic photosynthetic organisms [23], while an oxidized PQ-pool signals the state 2→1 fluorescence increase and a reduced PQ-pool the state 1→2 fluorescence decrease in the eukaryotic as well as the prokaryotic photosynthetic organisms ([24]; reviewed in [25–27]).

The photosynthetic apparatus of cyanobacteria differs significantly from that of green plants. Excitation energy transfer studies with wild-type cells [28] have indicated that the extrinsic phycobilisomes (PBS) of cyanobacteria can couple to photosystem (PS) II as well as to PS I. Proposals for state transition mechanisms were based either on mobile PBS [27], which changes its association with PSII and PSI, or on a "spillover" of Chl *a* excitation from PSII to PSI [29]. A hybrid model combining both spillover and PBS mobility has also been proposed [30,31].

The share of PBS excitation that PS II receives, and the intensity of the Chl *a* fluorescence it emits, can be regulated indirectly by modifying the PBS→PSI excitation transfers. There are two ways to achieve that. First by suspending cyanobacterial cells in strongly hyper-osmotic media (i.e., medium osmolarity >> cytoplasmic osmolarity). Suspension hyperosmolarity favours PBS→PS I excitation transfers and suppresses PBS→PS II transfers. Such cells are locked in the low fluorescence state 2 and cannot be light acclimated to the high fluorescence state 1 [reviewed by Papageorgiou and Stamatakis [32]. Second, by treating the cells with N-ethylmaleimide (NEM). This treatment blocks PBS→PS I excitation transfers and, correspondingly, enhances PBS→PS II transfers [33]. NEM-treated cells exist in a high fluorescence state (quasi-state 1) even in darkness [34].

In the present work we have investigated the changes in the Chl *a* fluorescence output at room temperature of cyanobacterium *Synechococcus* sp PCC 7942 focusing on the phenomenology and etiology of the fluorescence rise along SM rise and its subsequent decay along MT decay. We shall present evidence, based on the effects of electron and excitation transfer inhibitors, which shows that the SM fluorescence rise at room temperature is at least biphasic, containing contributions from the state 2 to state 1 transition and from an electron transport-dependent dequenching process, while the MT decline appears to be dominated by electron transport-dependent quenching.

2. Materials and methods

2.1. Cell cultures

Synechococcus sp. PCC 7942 cells were cultured in the medium BG11 [35], that contained additionally 20 mM HEPES NaOH, pH 7.5 (basal medium). The cultures were provided with white fluorescent light (100 μE m^{-2} s^{-1}) and 5% v/v CO_2 in air. Cells were harvested after 4 days (exponential phase), and they were suspended in basal medium at 20 μg Chla ml^{-1}.

2.2. Chemical treatments and sample preparations

Suspension media of defined osmolality consisted of sorbitol solutions in basal medium. Cyanobacteria are impermeable to sorbitol [36,37]. Osmolalities were measured cryoscopically as in [38]. The basal medium (80 mOsm kg^{-1}) was hypo-osmotic and the plus 0.8 M sorbitol medium was hyper-osmotic (1020 mOsm kg^{-1}). Osmolalities of cell suspensions were considered equal to those of the respective suspension media. Cells were treated with 0.1 M NEM for 40 min, as in [33]. Unreacted NEM was removed with two washes with basal medium.

2.3. Measurements of Chl a fluorescence

We measured Chl *a* fluorescence of cell suspensions with two different fluorometers: a continuous excitation PEA-fluorometer (PEA, Hansatech, King's Lynn, Norfolk, UK) and a modulated excitation-emission fluorometer (PAM; Heinz Walz, Effeltrich, Germany).

The PEA fluorometer provides continuous excitation at 650 nm (3000 μE m^{-2} s^{-1}; $\Delta\lambda$=22 nm). It detects fluorescence at wavelengths above 700 nm (50% transmission at 720 nm) and records it continuously from 10 μs to 2 min with data acquisition every 10 μs for the first 2 ms, every 1 ms between 2 ms and 1 s, and every 100 ms thereafter. The instrument allows repeated recordings, separated by recording lapses, during which the actinic illumination of the sample is not interrupted. To record the full FI kinetics (fast and slow induction) we employed 10 successive 2-min recordings, separated by 10-s intervals, hence the total illumination time was extended to 21.5 min. Measurements were performed on 80 μl of cyanobacteria suspension (40 μg Chla ml^{-1}) in 3 mm diameter vials. The depth of the sample was 3 mm and the diameter of the irradiated area 3 mm.

The PAM fluorometer allows control of sample temperature. It provides periodic excitation pulses (650 nm; $\Delta\lambda$=25 nm; 1.6 kHz; 1 μs flashes; 70 nE m^{-2} s^{-1}; hereafter called measuring light), and detects only synchronous Chl *a*

fluorescence signals at $\lambda > 690$ nm. Measuring light was absorbed both by Chl *a* and by PBS chromophores, so Chl *a* was excited both directly and indirectly. Actinic light was provided as continuous illumination from an incandescent source (3000 μE m^{-2} s^{-1}; KL1500 Electronic; Schott Glasswerke, Germany). Samples were shielded by a heat reflecting mirror (Oriel No. 5740; steady transmittance from 440 to 780 nm) and an orange glass filter (Corning CS 3-67).

3. Results

Fig. 1 displays a panoramic views of the full FI pattern of *Synechococcus* cells suspended in hypo-osmotic medium without DCMU (I; control) and with DCMU (II), as well as of cells suspended in hyper-osmotic medium without DCMU (III). DCMU and hyper-osmolarity have no appreciable effect on the OJI phases of the fast transient but they do clearly modify the FI pattern thereafter. Thus, the FI pattern of the +DCMU cells (curve II) has no P maximum, its minimum S occurs earlier (at ~1 s, compared to ~10 s of control cells), the SM rise is clearly biphasic and maximum M is lower (compared to an apparent monophasic rise to a higher M in control cells). Characteristically, also, the M maximum in the +DCMU cells is delayed by ~200 s compared to the M maximum of the −DCMU cells. Hyper-osmolarity eliminated not only the P maximum but also the entire SMT transient (curve III). In hyper-osmotic suspension, cells display a continuous decay of Chl *a* fluorescence, from J all the way to T, that includes inflections which are synchronous to the I, P and S features of control cells.

Physical movements of PBS have been implicated in state transitions in cyanobacteria [27,31]. To find out if they play also some role in FI we recorded kinetics of PBS-sensitized Chl *a* fluorescence using hypo-osmotic *Synechococcus* cell suspensions, without and with DCMU, after equilibrating them at 2 °C. According to Fig. 2, the low temperature affects only the slow induction phase, having no effect on the initial

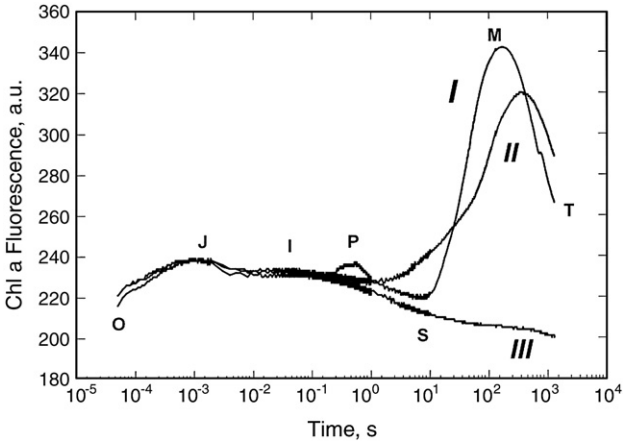

Fig. 1. Total FI kinetics (O–J–I–P–S–M–T), recorded at room temperature with cell suspensions of cyanobacterium *Synechococcus* sp PCC7942, and plotted against a logarithmic time scale. Trace I, cells suspended in hypo-osmotic medium (control cells); trace II, cells suspended in hypo-osmotic medium plus 20 μM DCMU; trace III, cells suspended in hyperosmotic medium. Measurements were made with the PEA fluorometer (Hansatech, UK). Cell suspensions were acclimated to darkness for 4 min prior to recording the FI kinetics.

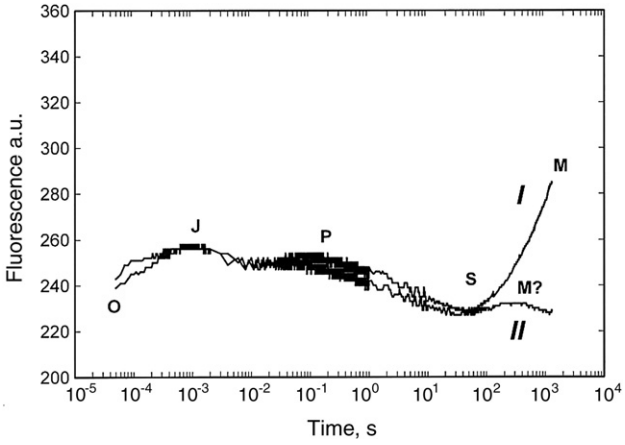

Fig. 2. Total FI kinetics (O–J–I–P–S–M) recorded with *Synechococcus* sp PCC7942 cells that had been equilibrated at 2 °C. Trace I, control cells; trace II, cells in the presence of 20 μM DCMU. Other details, as in the legend of Fig. 1.

fast transient. In both ±DCMU cells, the onset of the SM rise has been shifted to 60–70 s. In the +DCMU cells, the typical SMT rise of room temperature cells (Fig. 1, curve II; M above P) is replaced at low temperature with a shallow SMT transient (Fig. 2, curve II; M below P). The −DCMU cells, on the other hand, display an SM rise which, however, is much slower than that of the room temperature cells and does not reach a maximum within the 21.5 min time frame of these experiments (Fig. 2, curve I). This experiment suggests that the SM rise in cyanobacteria involves at least two processes: (i) a PBS-dependent fluorescence increase, most likely due to a state 2 to state 1 transition, which the low temperature inhibits, and (ii) an electron transport-dependent dequenching which is not inhibited at the low temperature (although it may be delayed).

In Fig. 3 experiment we extended the temperature study of the SM rise in ±DCMU cells, using the PAM fluorometer whose sample holder allowed us to equilibrate the cells at various temperatures. The questions asked here concerned the effects of various intermediate temperatures (between 30 °C and 2 °C) on the SM kinetics, and whether the effects of the low temperature treatments could be reversed by rewarming the cell suspensions. Fig. 3 displays the recorded the kinetics on linear time scales. Panel A shows that the SM rise is slower in the +DCMU cells than in the −DCMU cells, at all temperatures tested (i.e. 30, 16 and 10 °C), and the corresponding amplitudes smaller. Panel B displays SM kinetics of ±DCMU cells that had been subjected to 30 °C→2 °C→30 °C temperature cycles. It shows that in cells rewarmed to 30 °C, the SM fluorescence rise is restored, however at slower rise rates and with somewhat lower amplitudes.

Cyanobacterial cells treated with NEM are in a quasi-state 1 situation, even during dark adaptation, because the treatment blocks the PBS→PS II excitation transfer route and maximizes the PBS→PS I transfer route [33,34]. On the other hand, in hyperosmotic suspension, the cyanobacteria cells are locked in state 2, in which the PBS→PSI excitation transfer route is maximized and the PBS→PS II route is minimized. In hyper-osmotic suspensions the cyanobacteria cells cannot be light-

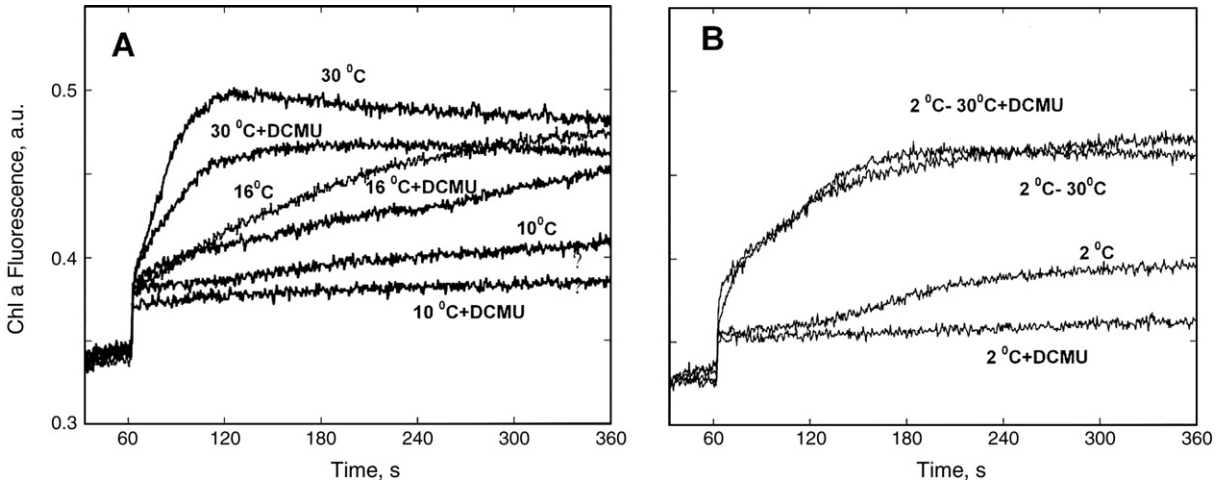

Fig. 3. Effects of cooling *Synechococcus* sp PCC7942 cell suspensions to near 0 °C temperatures on the FI kinetics, plotted against a linear time scale. (A) FI traces of ±DCMU cell suspensions equilibrated at different temperatures (indicated on the figure). (B) FI traces of ±DCMU cell suspensions that have been subjected to 30 °C→2 °C→30 °C temperature cycles. Measurements were made with a PAM fluorimeter.

acclimated to state 1 [32,34,36–38]. Essentially, both treatments deny the PBS of any regulatory function in the distribution of excitation energy to PS I and PS II.

In the Fig. 4 experiment we examined how the NEM-treatment and suspension hyperosmolarity influence the FI pattern of *Synechococcus*. The FI of a hypo-osmotic control suspension of untreated cells is included for comparison. In all NEM-treated preparations, the structured OJIP features of the fast transient of control cells were replaced by kinetic traces, lying much higher that the corresponding phases of the control cells and retaining the same, although, shallower features. The most conspicuous difference is that in the NEM-treated cells, the PSMT phases of the FI of the control cells have been replaced by a continuous, with some inflections, P→T decay of Chl a fluorescence. Another remarkable difference is that, at hyper-osmotic conditions, the entire FI curve of the NEM treated cells lies above the corresponding FI curve at hypo-osmotic conditions.

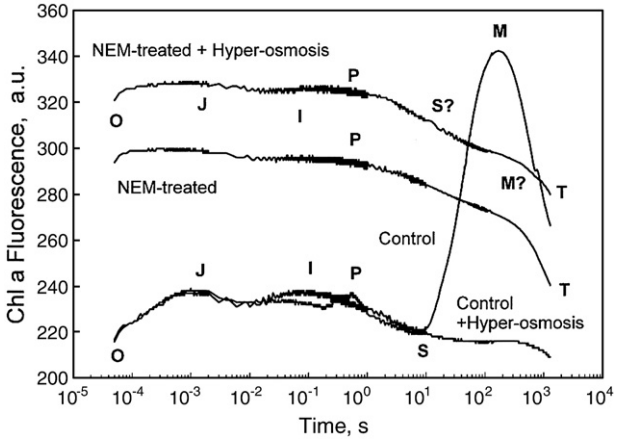

Fig. 4. Total FI kinetics (O–J–I–P–S–M–T) in *Synechococcus* sp PCC7942 cell suspensions. I, control cells; II, cells treated with N-ethyl maleimide (NEM), in hypo-osmotic or in hyper-osmotic suspension. Other details, as in the legend of Fig. 1.

4. Discussion

Total FI patterns of higher plants (see e.g., [18,38–45]) and algae (see e.g., [46–48]) are characteristically different from those of *Synechococcus*-like cyanobacteria (Fig. 1). In the former, the maximum fluorescence output occurs at P, with M being a minor maximum, or an inflection, or sometimes absent, while in cyanobacteria the maximum output occurs at M, with P now being a minor maximum or an inflection. We propose that this difference is caused by the long dark adaptation period that precedes FI recordings. Plants and algae emerge from it in the high fluorescence light state 1, while cyanobacteria emerge in the low fluorescence light state 2. Thus, in plants and algae the post-P phases of FI (i.e., PSMT) are dominated by a state 1→2 transition (fluorescence decrease), while in cyanobacteria they are dominated by a state 2→1 transition (fluorescence increase). These general kinetic trends are further modulated by photochemical and nonphotochemical quenching processes (qP, de-qP, qN, de-qN). Our results, complemented with information obtained from the literature, support this thesis.

Inhibition of the noncyclic PSET by DCMU elicits different response in plants and algae, on one hand, and in cyanobacteria on the other. In leaves [16,40], intact chloroplasts [49], protoplasts [22] and in algae [15,49], and by analogy in cyanobacteria, the PS decay reflects the combined contributions by photochemical (qP) and nonphotochemical quenching (qN; mostly acidification of the lumen), and possibly by a state 1→2 transition [22]. DCMU abolishes all FI phases beyond P in plants [15,50], and the PS decay in cyanobacteria, but it allows the major SMT transient to materialize (Fig. 1; also refs. [51–53]). Compared, however, to the −DCMU cyanobacterial cells, the SM rise in the +DCMU cells starts earlier (at ~1 s vs. ~10 s), is clearly biphasic, its amplitude is smaller, and its maximum M is delayed (to ~500 s vs. ~200 s). This phenomenology suggests that the earlier onset of the SM rise in the +DCMU cells must relate to

the elimination of the qP and qN contributions, to a faster oxidation of the PQ pool by PS I and, in consequence, to an earlier onset of the state 2→1 transition. It precludes, also, dominant contributions by qP and de-qP to the SMT transient.

The SM rise in higher plant leaves has been attributed predominantly to the reduction of Q_A (de-qP) and the suppression of transmembrane ΔpÇ (de-qN; refs. [16,18,40]) with a predominant contribution by de-qP [16]. Such de-qP and de-qN contributions to the SM rise are also likely for −DCMU cyanobacterial cells. However, the dominant contributor in these cells is likely the state 2→1 transition, as the PBS-sensitized fluorescence spectra at 77 K of *Synechococcus* show [31]. DCMU eliminates the non-cyclic qP but conceivably it allows qN (via proton translocation by cyclic PSET) and of course it allows a state 2→1 transition, as demonstrated earlier [51,53].

Only −DCMU *Synechococcus* cells display an SM fluorescence rise at 2 °C (Fig. 2), which, however, is delayed, incomplete and slower than the corresponding SM rise of room temperature cells (Fig. 1). At 2 °C, +DCMU cells display at 2 °C only a shallow P-to-T decay which is interrupted by a low SMT transient (M below P). Upon cooling cyanobacteria to ~0 °C, phycobiliprotein fluorescence is known to increase [54,55], and correspondingly, Chl a fluorescence to decrease [55].These changes, which were found to be reversible upon rewarming, have been interpreted in terms of a physical detachment of the PBS from the thylakoid membrane. This, according to T. Förster [56], would depress the PBS→PS II, PS I excitation transfers and the PBS-sensitized Chl *a* fluorescence and would enhance the PBS fluorescence. Actually, there is no rigorous proof of such cold-induced physical detachments. A slight orientation change between the interacting chromophores (Förster's κ factor) may cause the same effect. Our Fig. 3 experiment shows that at the end of a 30 °C→2 °Cv→30 °C cycle, the cold-induced changes are not fully reversible since the SM rise becomes slower, although its amplitude is nearly recovered. The discrepancy may be attributed (i) to the fact that we measured Chl a fluorescence instead of the PBS fluorescence measured as in [54], and (ii) because *Synechococcus*, which displays an unusual high phase transition temperature (~13 °C) of its membrane lipids, is more susceptible to cold inactivation than other cyanobacteria (including *Spirulina* of ref. [55]) whose membrane lipids experience thermotropic phase transitions below 0 °C (reviewed by Murata and Wada, ref. [57]).

State transition mechanisms in cyanobacteria involve excitonic coupling between phycobilins of the extrinsic PBS and Chls of intrinsic holochromes. Excitation transfer occurs via specialized APC core subunits, the ApcE protein for PS II [58], and the ApcD protein for PS I [59]. State transitions have been visualized to involve direct transfers from PBS to PS II and PS I (namely, PS II←PBS→PS I), indirect transfer to PS I via PS II (known as spillover; PBS→PS II→PS I) or both routes (hybrid model; [60]). Basically, the regulation is effected via the PBS→PS I excitation transfer route: when it is maximized, the PBS-sensitized Chl *a* fluorescence is low, i.e., the cells are in state 2; when it is minimized, the PBS→PS II route is enhanced and the PBS-sensitized Chl *a* fluorescence is high, i.e., the cells are in state 1.

We have investigated the slow FI phenomenology in *Synechococcus* under conditions that the regulation of the PBS→PS I excitation transfer was removed (Fig. 4). We used for this either NEM-treated cells, or cells in hyper-osmotic suspension. NEM-treated cells, in which the PBS→PS I excitation transfer is blocked while the PBS→PS II excitation transfer is enhanced, display the O(JI)P phases of FI at a higher level than the untreated controls, but not as high as the M maximum of the latter. This is indicative of a quasi-state 1 situation that was achieved not by light acclimation but chemically. Also, in the NEM-treated cells, the P-SMT pattern, observed with the control cells, is replaced with a continuous, plant-like, P→T fluorescence decay. Hyper-osmolarity, which maximizes the PBS→PS I and minimizes PBS→PS II excitation transfers (a quasi-state 2 situation), and it does not allow the light-acclimative transition to state 1, does not upshift the O(JI)P phases, but it does replace the SMT transient with a continuous P→T fluorescence decay.

The higher O(JI)P plateau of the +NEM cells, compared to control cells, can be rationalized in terms of a blocked PBS→PS I excitation transfer and an enhanced PBS→PS II excitation transfer, namely a state 1 situation. However, this argument cannot explain why the O(JI)P plateau of the +NEM cells is well below the M level of the control cells, and why in hyper-osmotic suspension the O(JI)P transient of the +NEM cells lies higher than that of the hypo-osmotic suspension. Part of the state 2→1 transition during SM, therefore, may not be regulated by the PBS. There is substantial phenomenological evidence for such "spillover" type regulation but no rigorous physical explanation has been proposed [29,60].

An intriguing consequence of eliminating the regulation of the excitation the PBS→PS I excitation transfer route, and indirectly the regulation of excitation transfer to PS II by NEM or by hyper-osmosis is the conversion of the typical cyanobacterial FI kinetics to typical plant-like FI kinetics, represented. Fig. 5 illustrates the similarity of the latter cyanobacterial FI pattern with that of a higher plant.

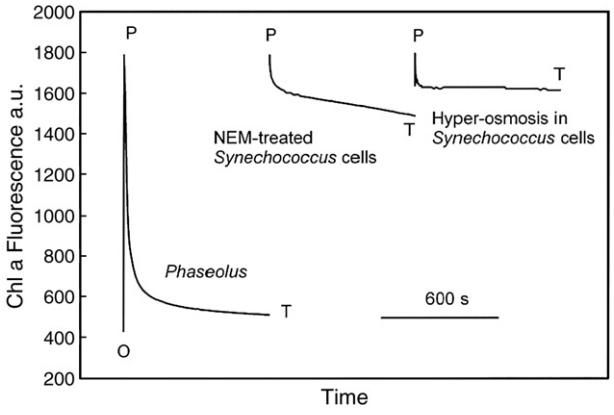

Fig. 5. Total FI kinetics of recorded with a *Phaseolus* leaf, a hypo-osmotic suspension of NEM-treated *Synechococcus* cells and a hyper-osmotic suspension of control Synechococcus cells. The kinetics have been recorded at identical conditions and are displayed against linear time scales.

The phenomenology of the MT decay phase of the total Chl a fluorescence induction pattern has been studied mostly in plants (leaves, protoplasts, intact chloroplasts and broken chloroplasts), less in algae and little, if any at all, in the cyanobacteria. In higher plants and algae, the SMT transient is a minor episode, often absent, during the more extensive PT decay to which the state 1→2 fluorescence lowering [22] makes a substantial contribution along with qP and qN fluorescence quenching processes [16,18,22,61,62]. During SM and during most of MT, the rates of photosynthetic O_2 evolution [50,62] and CO_2 uptake [63] rise and become steady at about T. At nonphysiological CO_2 and O_2 levels in the gas phase above a leaf, multiple damped repetitions of the SMT fluorescence transient (oscillations) and corresponding antiparallel and slightly shifted (~10 s) oscillations of the O_2 evolution rate have been recorded [64,65].

Comparable data for cyanobacteria do not exist and is highly questionable whether we can obtain insight by using analogies. For one thing, a plant leaf is near, or at state 2 when the MT fluorescence decay takes place, while a cyanobacterium is in state 1 and probably stays there being continuously illuminated during MT decay. A clue may be sought in the fact that the MT decay in cyanobacteria materializes only under strong actinic illumination, so perhaps photoinhibitory effects may be involved. A preliminary study, in which we recorded repetitive FI patterns, separated by a 10-min dark interval, revealed no photoinhibitory effects up to 1500 μÅ m^{-2} s^{-1} actinic light intensities. The etiology of the MT decay in cyanobacteria remains an open question.

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References

[1] H. Kautsky, Hirsch, Neue Versuche zur Kohlensäureassimilation, Naturwissensch 48 (1931) 964.
[2] Govindjee, G. Papageorgiou, Chlorophyll fluorescence and photosynthesis: Fluorescence transients, in: A.C. Giese (Ed.), Photophysiology, vol. 6, Academic Press, NewYork, 1971, pp. 1–46.
[3] P. Mohanty, Govindjee, The slow decline and the subsequent rise of chlorophyll fluorescence transients in intact algal cells, Plant. Biochem. J. 1 (1974) 78–106.
[4] G.C. Papageorgiou, Chlorophyll fluorescence: an intrinsic probe of photosynthesis, in: Govindjee (Ed.), Bioenergetics of Photosynthesis, Academic Press, New York, 1975, pp. 319–372.
[5] Govindjee, Sixty-three years since Kautsky: Chlorophyll *a* fluorescence, Aust. J. Plant Physiol. 22 (1995) 131–160.
[6] Govindjee, Chlorophyll fluorescence. A bit of basics and history, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration, Vol. 19, Chlorophyll Fluorescence: A Signature of Photosynthesis, Springer, Dordrecht, The Netherlands, 2004, pp. 1–42.
[7] G.C. Papageorgiou, Govindjee (Eds.), Chlorophyll *a* Fluorescence: A Signature of Photosytnthesis, Springer, Dordrecht, The Netherlands, 2004.
[8] P. Bonaventura, J. Myers, Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*, Biochim. Biophys. Acta 189 (1969) 366–383.
[9] N. Murata, Control of excitation transfer in photosynthesis I. Light-induced change of chlorophyll *a* fluorescence in *Porhyridium cruentum*, Biochim. Biophys. Acta 172 (1969) 242–251.
[10] L.N.M. Duysens, H.E. Sweers, Mechanism of two photochemical reactions in algae as studied by means of fluorescence in: Japanese Society of Plant Physiologists (Eds.), Studies on Microalgae and Photosynthetic Bacteria, University of Tokyo Press, 1963, pp. 353–372.
[11] S. Malkin, Fluorescence induction studies in isolated chloroplasts II, kinetic analysis of the fluorescence intensity dependence on time, Biochim. Biophys. Acta 126 (1966) 433–442.
[12] S. Malkin, B. Kok, Fluorescence induction studies in isolated chloroplasts. I. Number of components involved in the reaction and quantum yields, Biochim. Biophys. Acta 126 (1966) 413–432.
[13] H.J. van Gorkom, J.J. Taminga, J. Haveman, Primary reactions, plastoquinone and fluorescence yield in subchloroplast fragments with deoxycholate, Biochim. Biophys. Acta 347 (1974) 417–438.
[14] C. Vernotte, A.L. Etienne, J.M. Briantais, Quenching of the system II chlorophyll fluorescence by the plastoquinone pool, Biochim. Biophys. Acta 545 (1979) 519–527.
[15] G.H. Krause, C. Vernotte, J.M. Briantais, Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae, resolution into two components, Biochim. Biophys. Acta 679 (1982) 116–124.
[16] M. Bradbury, N.R. Baker, A quantitative determination of photochemical and non-photochemical quenching during the slow phase of the chlorophyll fluorescence induction curve of bean leaves, Biochim. Biophys. Acta 765 (1984) 275–281.
[17] J.M. Briantais, C. Vernotte, G.H. Krause, E. Weis, Chlorophyll *a* fluorescence of higher plants: Chloroplasts and leaves, in: Govindjee, J. Amesz, D.C. Fork (Eds.), Light Emission by Plants and Bacteria, Acad Press, New York, 1986, pp. 539–583.
[18] P. Horton, Effects of changes in the capacity for photosynthetic electron transfer and photophosphorylation on the kinetics of fluorescence induction in isolated chloroplasts, Biochim. Biophys. Acta 724 (1983) 404–410.
[19] M.N. Sivak, D.A. Walker, Some effects of CO_2 concentration and decreased O_2 concentration on induction fluorescence in leaves, Proc. R. Soc. Lond., B Biol. Sci. 217 (1983) 377–392.
[20] P. Horton, Relations between electron transport and carbon dioxide assimilation; simultaneous measurement of chlorophyll fluorescence. Transthylakoid pH gradients and O_2 evolution I isolated chloroplasts, Proc. R. Soc. Lond., B 217 (1983) 405–416.
[21] W.J. Vredenberg, Chlorophyll *a* fluorescence induction and changes in the electrical potential of the cellular membranes of green plant cells, Biochim. Biophys. Acta 223 (1970) 230–239.
[22] P. Horton, A. Hague, Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching, Biochim. Biophys. Acta 932 (1988) 107–115.
[23] N.E. Holt, G.R. Fleming, K.K. Niyogi, Toward an understanding of nonphotochemical quenching in green plants, Biochemistry 43 (2004) 8281–8289.
[24] C.W. Mullineaux, J.F. Allen, State 1–state 2 transitions in the cyanobacterium *Synechococcus* 6301 are controlled by the redox state of electron carriers between Photosystems I and II, Photosynth. Res. 23 (1989) 297–311.
[25] W.P. Williams, J.F. Allen, State 1 / state 2 changes in higher plants and algae, Photosynth. Res. 13 (1987) 19–45.
[26] J.F. Allen, J. Forsberg, Molecular recognition in thylakoid structure and function, Trends Plant Sci. 6 (2001) 317–326.
[27] J.F. Allen, C.W. Mullineux, Probing the mechanism of state transitions in oxygenic photosynthesis by chlorophyll fluorescence spectroscopy. Kinetics and imaging, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration, Vol. 19, Chlorophyll Fluorescence: A Signature of Photosynthesis, Springer, Dordrecht, The Netherlands, 2004, pp. 447–461.
[28] C.W. Mullineaux, Excitation energy transfer from phycobilisomes to Photosystem I in a cyanobacterium, Biochim. Biophys. Acta 1100 (1992) 285–292.

[29] D. Bruce, S. Brimble, D.A. Bryant, State transitions in a phycobilisomes less mutant of the cyanobacterium *Synechococcus* sp. PCC 7002, Biochim. Biophys. Acta 974 (1989) 66–73.
[30] D. Bruce, S. Vasil'ev, Excess light stress: Multiple dissipative processes of excess excitation, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration, Vol. 19, Chlorophyll *a* Fluorescence. A Signature of Photosynthesis, Springer, Dordrecht, The Netherlands, 2006, pp. 497–523.
[31] M. Koblízek, J. Komenda, J. Masojídek, State transitions in the cyanobacterium *Synechococcus* PCC 7942. Mobile antenna or spillover? in: G. Garab (Ed.), Photosynthesis: Mechanisms and Effects, vol. 1, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998, pp. 213–216.
[32] G.C. Papageorgiou, K. Stamatakis, Water and solute transport in cyanobacteria as probed by chlorophyll fluorescence, in: G.C. Papageorgiou, Govindjee (Eds.), Advances in Photosynthesis and Respiration, Vol. 19, Chlorophyll α Fluorescence. A Signature of Photosynthesis, Springer, Dordrecht, The Netherlands, 2006, pp. 663–678.
[33] A.N. Glazer, Y.M. Gindt, C.F. Chan, K. Sauer, Selective disruption of energy flow from phycobilisomes to photosystem I, Photosynth. Res. 40 (1994) 167–173.
[34] K. Stamatakis, G.C. Papageorgiou, The osmolality of the cell suspension regulates phycobilisome-to-photosystem I excitation transfers in cyanobacteria, Biochim. Biophys. Acta 1506 (2001) 172–181.
[35] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.T. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.
[36] G.C. Papageorgiou, A. Alygizaki-Zorba, A sensitive method for the estimation of the cytoplasmic osmolality of cyanobacterial cells using chlorophyll a fluorescence, Biochim. Biophys. Acta 1335 (1997) 1–4.
[37] G.C. Papageorgiou, A. Alygizaki-Zorba, N. Ladas, N. Murata, A method to probe the cytoplasmic osmolality and water and solute fluxes across the cell membrane of cyanobacteria with chlorophyll *a* fluorescence: experiments with *Synechococcussp*. PCC 7942, Physiol. Plant. 103 (1998) 215–224.
[38] K. Stamatakis, N.P. Ladas, A. Alygizaki-Zorba, G.C. Papageorgiou, Sodium chloride-induced volume changes of freshwater cyanobacterium *Synechococcus* sp. PCC 7942 can be probed by chlorophyll *a* fluorescence, Arch. Biochem. Biophys. 370 (1999) 240–249.
[39] U. Schreiber, W. Vidaver, V.C. Runeckles, P. Rosen, Chlorophyll fluorescence assay for ozone injury in intact plants, Plant Physiol. 61 (1978) 80–84.
[40] M. Bradbury, N.R. Baker, Analysis of the slow phases of the in vivo chlorophyll fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystems I and II, Biochim. Biophys. Acta 635 (1981) 542–551.
[41] R.T. Furbank, D.A. Walker, Photosynthetic induction in C 4 leaves. An investigation using infra-red gas analysis and chlorophyll *a* fluorescence, Planta 163 (1985) 75–83.
[42] A.A. Bulychev, M.M. Niyazova, V.B. Turovetsky, Evidence for the delayed photoactivation of electrogenic electron transport in chloroplast membranes, Biochim. Biophys. Acta 808 (1985) 186–191.
[43] N.G. Bukhov, P. Mohanty, M.G. Rakhimberdieva, N.V. Karapetyan, Analysis of dark-relaxation kinetics of variable fluorescence in intact leaves, Planta 187 (1992) 122–127.
[44] V.A. Karavaev, I.B. Polyakova, M.K. Solntsev, T.P. Yurina, Effect of various chemical agents on photosynthesis studied by the method of fluorescence induction, J. Lumin. 76 and 77 (1998) 335–338.
[45] Govindjee, P. Spilotro, An *Arabidopsis thaliana* mutant, altered in the γ-subunit of ATP synthase, has a different pattern of intensity-dependent changes in non-photochemical quenching and kinetics of the P-to-S fluorescence decay, Funct. Plant Biol. 29 (2002) 425–434.
[46] K. Satoh, D.C. Fork, A new mechanism for adaptation to changes in light intensity and quality in the red alga, *Porphyra perforata* I. Relation to state 1–state 2 transitions, Biochim. Biophys. Acta 722 (1983) 190–196.
[47] Govindjee, M.J. Seufferfeld, Non-photochemical quenching of chlorophyll *a* fluorescence: early history and characterization of two xanthophyll cycle mutants of *Chlamydomonas reinhardtii*, Funct. Plant Biol. 29 (2002) 1141–1155.
[48] G. Paresys, C. Rigart, B. Rousseau, A.W.M. Wong, F. Fan, J.-P. Barbier, J. Lavaud, Quantitative and qualitative evaluation of phytoplankton communities by trichromatic chlorophyll fluorescence excitation with special focus on cyanobacteria, Water Res. 39 (2005) 911–921.
[49] G.H. Krause, J.M. Briantais, C. Vernotte, Characterization of chlorophyll fluorescence quenching in chloroplasts by fluorescence spectroscopy at 77 K. I. ΔpH-dependent quenching, Biochim. Biophys. Acta 723 (1983) 169–175.
[50] G.C. Papageorgiou, Govindjee, Light-induced changes in the fluorescence yield of chlorophyll *a* in vivo. II. *Chlorella pyrenoidosa*, Biophys. J. 8 (1968) 1316–1328.
[51] G.C. Papageorgiou, Govindjee, Changes in intensity and spectral distribution of fluorescence. Effect of light treatment on normal and DCMU*-poisoned *Anacystis nidulans*, Biophys. J. 7 (1967) 376–389.
[52] G.C. Papageorgiou, Govindjee, Light-induced changes in the fluorescence yield of chlorophyll *a* in vivo I. *Anacystis nidulans*, Biophys. J. 8 (1968) 1301–1315.
[53] P. Mohanty, Govindjee, Light-induced changes in the fluorescence yield of chlorophyll *a* in *Anacystis nidulans* I. Relationship of slow fluorescence changes with structural changes, Biochim. Biophys. Acta 305 (1973) 95–104.
[54] U. Schreiber, Reversible uncoupling of energy transfer between phycobilins and chlorophyll in *Anacystis nidulans*. Light stimulation of cold induced phycobilisome detachment, Biochim. Biophys. Acta 591 (1980) 361–371.
[55] Y. Li, J.P. Zhang, J. Xie, J.Q. Zhao, L.J. Jiang, Temperature-induced decoupling of phycobilisomes from reaction centers, Biochim. Biophys. Acta 1504 (2001) 229–234.
[56] T. Förster, Fluoreszenz Organischer Verbindungen, Vandenhoeck and Ruprecht, Göttingen, 1951.
[57] N. Murata, H. Wada, Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria, Biochem. J. 308 (1995) 1–8.
[58] Y.M. Gindt, J. Zhou, D.A. Bryant, K. Sauer, Spectroscopic studies of phycobilisome subcore preparations lacking key core chromophores: assignment of excited state energies to the Lcm, beta 18 and alpha AP-B chromophores, Biochim. Biophys. Acta 1186 (1994) 153–162.
[59] P. Maxson, K. Sauer, J.H. Zhou, D.A. Bryant, A.N. Glazer, Spectroscopic studies of cyanobacterial phycobilisomes lacking core polypeptides, Biochim. Biophys. Acta 977 (1989) 40–51.
[60] M.D. McConnell, R. Koop, S. Vasil'ev, D. Bruce, Regulation of the distribution of chlorophyll and phycobilin absorbed excitation energy in cyanobacteria. A structure-based model for the light state transition, Plant Physiol. 130 (2002) 1201–1212.
[61] M.N. Sivak, R.T. Prinsley, D.A. Walker, Some effects of changes in gas-phase on the steady-state chlorophyll *a* fluorescence exhibited by illuminated leaves, Proc. R. Soc., B 217 (1983) 393–404.
[62] M.N. Sivak, K.J. Dietz, U. Heber, D.A. Walker, The relationship between light scattering and chlorophyll *a* fluorescence during oscillations in photosynthetic carbon assimilation, Arch. Biochem. Biophys. 237 (1985) 513–519.
[63] T.T. Bannister, G. Rice, Parallel time courses of oxygen evolution and chlorophyll fluorescence, Biochim. Biophys. Acta 162 (1968) 555–580.
[64] C.R. Ireland, S.P. Long, N.R. Baker, The relationship between carbon dioxide fixation and chlorophyll *a* fluorescence during induction of photosynthesis in maize leaves at different temperatures and carbon dioxide concentrations, Planta 100 (1984) 550–558.
[65] D.A. Walker, M.N. Sivak, R.T. Prinsley, J.K. Cheesbrough, Simultaneous measurement of oscillations in oxygen evolution and chlorophyll *a* fluorescence in leaf pieces, Plant Physiol. 73 (1983) 542–549.