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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*