The occurrence of rapidly reversible non-photochemical quenching of chlorophyll *a* fluorescence in cyanobacteria

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Abstract Cyanobacteria have previously been considered to differ fundamentally from plants and algae in their regulation of light harvesting. We show here that in fact the ecologically important marine prochlorophyte, *Prochlorococcus*, is capable of forming rapidly reversible non-photochemical quenching of chlorophyll *a* fluorescence (NPQf or qE) as are freshwater cyanobacteria when they employ the iron stress induced chlorophyll-based antenna, IsiA. For *Prochlorococcus*, the capacity for NPQf is greater in high light-adapted strains, except during iron starvation which allows for increased quenching in low light-adapted strains. NPQf formation in freshwater cyanobacteria is accompanied by deep F_o quenching which increases with prolonged iron starvation.

Keywords: Non-photochemical quenching; Light harvesting; Cyanobacteria; Photoprotection; *Prochlorococcus*

1. Introduction

Unicellular cyanobacteria of the closely related genera *Prochlorococcus* and *Synechococcus* are broadly distributed in oceanic waters [1] and contribute significantly to global primary production [2]. The in situ community structure of these organisms is complex, with specific ecotypes occupying different niches [3–5]. Despite being closely related, the widespread distribution of *Prochlorococcus* in the open oceans and throughout the entire euphotic water column ensures that this genus is far more abundant than *Synechococcus*, which are typically restricted to surface waters. The success of *Prochlorococcus* is due to the presence of genetically distinct high light (HL) and low light (LL)-adapted ecotypes, which exhibit particular depth-dependent distributions. Recently, a comparison of the newly sequenced genomes of two *Prochlorococcus* strains, representative of HL (MED4) and LL (MIT9313)-adapted lineages, with that of a marine *Synechococcus* strain (*Synechococcus* sp. WH8102) highlighted the occurrence of distinct light harvesting systems as the key feature defining the two genera and therefore dictating niche distribution in the oceans [6].

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Abbreviations: NPQ, non-photochemical quenching; NPQf, rapidly reversible non-photochemical quenching; HL, high light; LL, low light; PSII, photosystem II; PSI, photosystem I; PAM, pulse amplitude modulated

The Pcb light-harvesting antenna binds divinyl chlorophylls *a* and *b* [7] and distinguishes the photosynthetic apparatus of *Prochlorococcus* from other cyanobacteria, which employ the non-chlorophyll-based phycobilisome. It still remains unclear, however, how the Pcb antenna of *Prochlorococcus* confers the differences in photosynthetic physiology that account for the niche partitioning observed in the oceans, particularly with respect to the unique success of *Prochlorococcus* over a wide range of depths.

The Pcb light harvesting antenna proteins form a super family along with the cyanobacterial antenna protein, IsiA, and the photosystem II (PSII) core protein CP43 [8]. Under different stress conditions, including iron depletion, some cyanobacteria, most notably the freshwater *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803, rapidly synthesise the membrane-integral chlorophyll-binding antenna, IsiA [9,10]. The genome sequence of the marine cyanobacterial strain *Synechococcus* sp. WH8102 does not, however, contain the *isiA* gene. Recently, it was shown that during prolonged iron starvation of *Synechocystis* sp. PCC6803, a significant proportion of accumulated IsiA polypeptides are present in excess of those required for light harvesting [11]. It has been suggested that the additional IsiA pigment–protein complexes serve to protect PSII from excess excitation through the dissipation of light energy. A similar role for IsiA in photoprotection has also been suggested for iron starved *Synechococcus* sp. PCC7942 [12,13].

The dissipation of excess excitation energy within the chlorophyll antenna of higher plants and algae is well documented (reviewed in [14]). The dissipation of absorbed quanta as heat in the antenna gives rise to a strong light-dependent quenching of chlorophyll *a* fluorescence, which cannot be ascribed to the quenching caused by photochemistry. This quenching is therefore known as non-photochemical quenching (NPQ). Several processes contribute to NPQ in higher plants, however, the main process, known as NPQf or qE, requires a *trans*-thylakoid pH gradient and therefore reverses rapidly in the dark.

In contrast, cyanobacteria have so far been considered as unable to form NPQf. Instead, cyanobacteria are believed to regulate their light harvesting through the state transition, which involves movement of the membrane peripheral phycobilisome antenna complex between PSI and PSII in order to readdress imbalances in photosynthetic electron transport [15]. Recently, however, the freshwater cyanobacterium *Synechocystis* sp. PCC6803 has been shown to form NPQf when expressing the chlorophyll-based antenna IsiA [16]. Here, we show that the freshwater cyanobacterium *Synechococcus* sp. PCC7942 also forms NPQf when expressing the IsiA antenna, suggesting that this phenomenon may be widespread amongst

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cyanobacteria. In addition, we show that the ecologically important marine prochlorophyte, *Prochlorococcus*, which permanently possesses the chlorophyll-based Pcb antenna, is also capable of forming NPQf. The implications for niche partitioning of *Prochlorococcus* are discussed.

2. Materials and methods

2.1. Growth conditions

Prochlorococcus sp. strains PCC9511 and SS120 were grown in PCR-S11 liquid media as described previously [17]. *Synechococcus* sp. strain WH8102 was grown in ASW liquid media as described previously [18]. Cells were grown in continuous white light at 10 μmol quanta m^{-2} s^{-1} at a constant growth temperature of 25 °C. *Synechocystis* sp. PCC6803 and *Synechococcus* sp. PCC7942 were grown in liquid BG11 medium as described elsewhere [19]. Cells were grown in continuous white light at 30 μmol quanta m^{-2} s^{-1} at a constant growth temperature of 30 °C. Iron deplete cells for all strains were grown as described above but in the absence of iron containing compounds. Cells were iron starved for a total of 20 days. Following the initial transfer into iron free media, cells were subsequently transferred to fresh media every 4 days.

2.2. Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence measurements were recorded using a Hansatech FMS-1 fluorometer (Hansatech Instruments Ltd., Norfolk, UK). Cells were suspended at a chlorophyll concentration of approximately 2 μg ml^{-1} and maintained at a temperature equivalent to the respective growth temperature. All samples were dark adapted for 20 min prior to measurement. Saturating pulses were provided with white light at 3000 μmol quanta m^{-2} s^{-1} for 1 s durations. Actinic irradiance was provided with white light at the appropriate irradiance. NPQf was determined from the relaxation kinetics of chlorophyll *a* fluorescence in the dark as described previously [20].

3. Results

3.1. Chlorophyll a fluorescence quenching in marine cyanobacteria

Fig. 1A shows a typical chlorophyll *a* fluorescence trace for *Synechococcus* sp. strain WH 8102 measured using pulse amplitude modulated (PAM) fluorometry. Following exposure to moderate irradiance (300 μmol quanta m^{-2} s^{-1}) the fluorescence yield increases rapidly. This increase reflects the well-documented state transition in which the phycobilisome migrates from PSI (state II) in the dark to PSII (state I) in the light [21]. At a saturating irradiance of 1500 μmol quanta m^{-2} s^{-1} (Fig. 1B), a light-dependent decrease in fluorescence yield is observed. This fluorescence quenching is not, however, accompanied by rapid reversal of variable fluorescence in the dark and most likely represents the accumulation of photoinhibited PSII reaction centres.

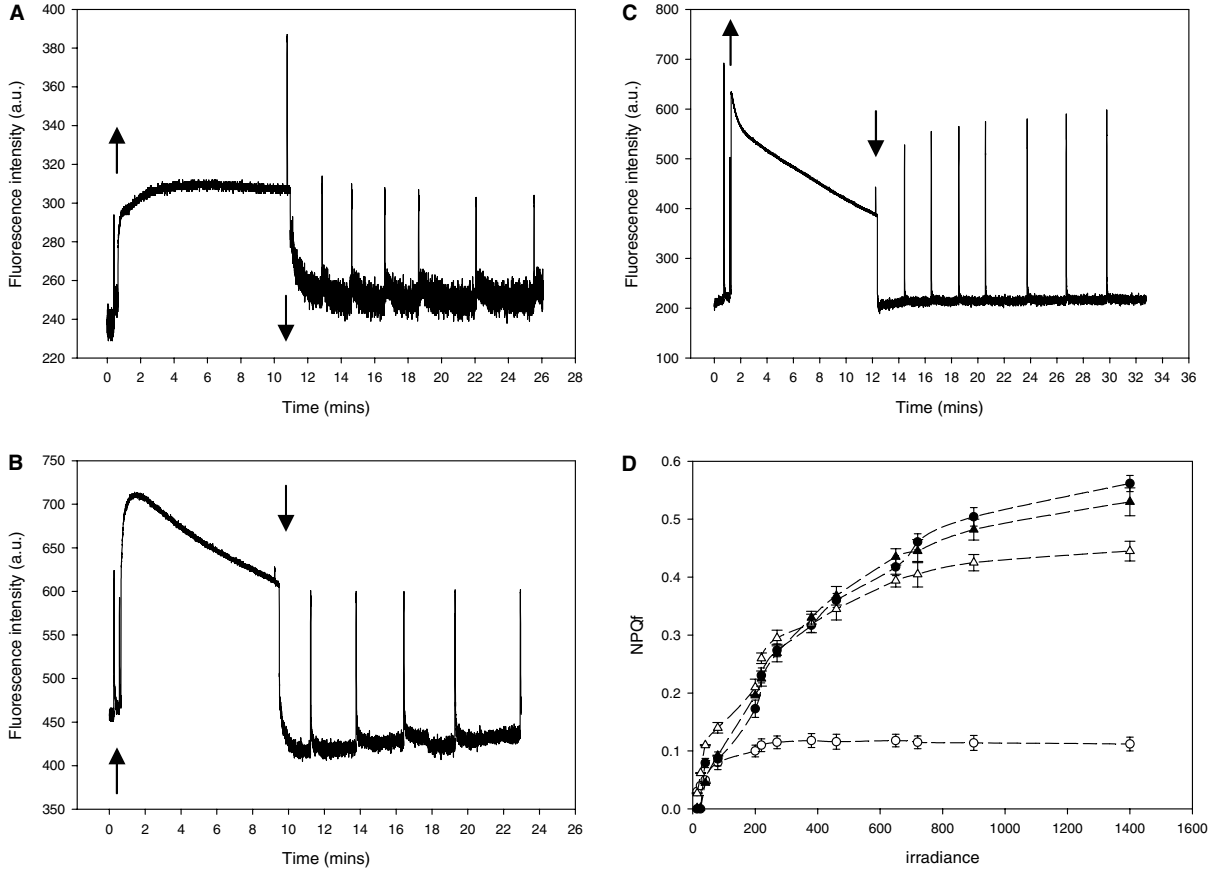

Fig. 1. Light induced changes in fluorescence yield and relaxation in the dark for cells of marine cyanobacteria. (A) *Synechococcus* sp. WH8102 with actinic light treatment at 300 μmol quanta m^{-2} s^{-1}. (B) *Synechococcus* sp. WH8102 with actinic light treatment at 1500 μmol quanta m^{-2} s^{-1}. (C) *Prochlorococcus* sp. PCC9511 with actinic light treatment at 800 μmol quanta m^{-2} s^{-1}. (D) The relationship between actinic irradiance and NPQf for *Prochlorococcus* sp. PCC9511 (closed circles represent iron replete cells, closed triangles represent iron deplete cells) and *Prochlorococcus* sp. SS120 (open circles represent iron replete cells, open triangles represent iron deplete cells). Continuous actinic irradiance is marked with ↑for on and with ↓ for off.