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Short communication

Dissipation of excess energy triggered by blue light in cyanobacteria with CP43′ (*isiA*)

Jean-Charles Cadoret[a,1], Raphaël Demoulière[a,1], Johann Lavaud[b], Hans J. van Gorkom[c], Jean Houmard[a,*], Anne-Lise Etienne[a]

[a]*Organismes Photosynthétiques et Environnement, FRE 2433 CNRS, Département de Biologie, ENS, 46 rue d'Ulm, 75230 Paris cedex 05, France*
[b]*Plant Ecophysiology, Department of Biology, University of Konstanz, Postfach M611, 78457 Konstanz, Germany*
[c]*Department of Biophysics, Leiden University, PO Box 9504, 2300 RA Leiden, Netherlands*

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Abstract

The chlorophyll-protein CP43′ (*isiA* gene) induced by stress conditions in cyanobacteria is shown to serve as an antenna for Photosystem II (PSII), in addition to its known role as an antenna for Photosystem I (PSI). At high light intensity, this antenna is converted to an efficient trap for chlorophyll excitations that protects system II from photo-inhibition. In contrast to the 'energy-dependent non-photochemical quenching' (NPQ) in chloroplasts, this photoprotective energy dissipation in cyanobacteria is triggered by blue light. The induction is proportional to light intensity. Induction and decay of the quenching exhibit the same large temperature-dependence.

Keywords: Non-photochemical quenching; Iron-stress; Chlorophyll antenna; Photoprotection; Photosystems

Sunlight is the driving force for photosynthesis. At the level of individual cells, the available light intensity varies unpredictably. Photosynthetic organisms can adjust to changes in average light intensity in various ways, together called photo-acclimation. They also have to cope with light intensity fluctuations that are too large or too fast for photo-acclimation to occur [1]. This is especially clear in the case of the oxygen-evolving Photosystem II (PSII), where over-saturating excitation leads to a rapid inhibition when destruction of the reaction center protein D1 becomes faster than its replacement [2]. In the chloroplasts of algae and higher plants, excessive illumination induces a change in the chlorophyll-binding antenna proteins that accelerates thermal dissipation of the excitation energy, as conveniently monitored by a decrease of the chlorophyll fluorescence yield [3]. This 'non-photochemical quenching' (NPQ) appears to be triggered by excessive acidification of the thylakoid lumen when consumption of the proton gradient by ATP synthesis cannot keep up with its generation by electron transport. It involves de-epoxidation of xanthophylls bound to the antenna proteins [4,5] and requires specific polypeptides, like the higher plant PsbS [6]. In some cases, NPQ can reduce the excitation rate of PSII reaction centers by an order of magnitude [7].

A comparable photoprotective mechanism has not been reported for cyanobacteria whose shorter generation time allows for a more rapid photo-acclimation [8]. Most species harvest light through phycobilisomes instead of chlorophyll-binding antenna proteins. Under stress conditions, however, many cyanobacteria such as *Synechococcus* PCC 7942 and *Synechocystis* PCC6803 synthesize a chlorophyll-binding polypeptide called CP43′, the *isiA* product [9–11]. CP43′ is a homolog of the PSII core antenna protein CP43 (PsbC) and of *Prochlorococcus* and *Prochloron* Pcbs, which serve as a peripheral antenna for both Photosystem I (PSI) and

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; NPQ, non-photochemical quenching; PSII, Photosystem II; PSI, Photosystem I; Q_A, PSII primary electron acceptor

* Corresponding author. Tel.: +33 1 4432 3519; fax +33 1 4432 3935.
E-mail address: jhoumard@biologie.ens.fr (J. Houmard).

[1] These two authors have contributed equally to this work.

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PSII [12,13]. CP43′ was recently shown to be an antenna for PSI [14,15]. A role as a PSII antenna has also been proposed [16]. However, in *Synechococcus* PCC 7942, the effective cross-section of PSII was actually found to be smaller in the presence of CP43′, suggesting that CP43′ is an efficient quencher of chlorophyll excitations that protects PSII from over-excitation [17,18].

It seems unlikely that the same protein could serve both as an antenna and as an excitation sink, unless it can exist in a non-quenching as well as in a quenching form. Here we demonstrate that the accumulation of CP43′ is, in fact, accompanied by the appearance of a reversible, light-induced NPQ that provides an effective protection against photo-inhibition of PSII. A first characterization of induction and decay of this cyanobacterial NPQ reveals that its regulation completely differs from the analogous phenomenon in the chloroplasts of photosynthetic eukaryotes.

Synechocystis PCC 6803 (Pasteur culture collection) was grown photo-autotrophically in 100 ml batch cultures at 32 °C in BG11 medium at a light intensity of 70 μE m^{-2} s^{-1}. For iron starvation, cells in their late exponential phase were diluted five times in iron-depleted BG11 medium, and transferred every other day into fresh iron-less medium, up to 10 days after the onset of starvation. The growth rate slowed down during iron starvation (0.7 of the initial rate after 4 days down to 0.3 after 10 days). PSI depletion and CP43′ accumulation were monitored by measurement of the fluorescence emission spectrum at 77 K in a Hitachi spectrofluorometer with excitation at 440 nm. Samples (20 μl) were rapidly vacuum-sucked onto a paper filter that was immediately plunged in liquid nitrogen. Light-induced chlorophyll fluorescence yield changes at room temperature were measured using a PAM-101 Walz fluorometer (Walz, Effletrich, Germany). Samples at a concentration of 5 (μg chlorophyll) ml^{-1} were dark-adapted for 10 min before measurement. Fluorescence induction kinetics in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) were performed as described in Ref. [19]. DCMU (200 μM final concentration) was added to a dark-adapted sample 15 min before measurement. Light-induced oxygen evolution was measured with a Clark electrode (Hansatech, King's Lynn, UK).

Iron-starvation of *Synechocystis* PCC 6803 leads to gradual changes over generations in the relative concentrations of CP43′, PSI and PSII. Especially the iron-rich PSI is severely down-regulated, while CP43′ is up-regulated [10]. To monitor the changing photosystem composition, fluorescence emission spectra were measured at 77 K with preferential chlorophyll excitation. The PSI peak was shifted from 723 to 719 nm and decreased relative to the amplitude of the PSII emission peaks at 685 and 695 nm, reflecting a decrease in the fraction of chlorophyll associated with PSI. The F685/F695 ratio increased from less than 1 in the control cells to 1.5 after 4 days of iron starvation and up to 2 after 10 days. Thus, CP43′ not only contributes to PSI excitation but also to PSII and ultimately accumulates in a

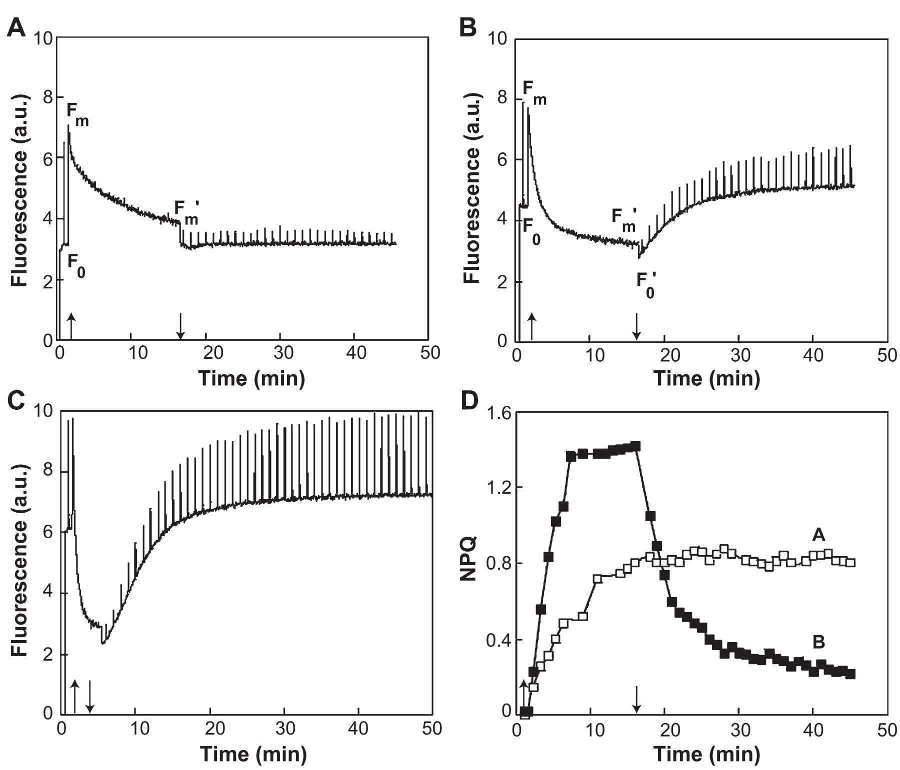

Fig. 1. Chlorophyll fluorescence yield changes induced by 15 min illumination with white light at 2 mE m^{-2} s^{-1}. The spikes at 1-min intervals result from flash-induced saturation to probe the yield in the absence of photochemical quenching. (A) Control cells; (B) 4-days iron-starved; (C) 10-days iron-starved; (D) NPQ kinetics ($=F_m/F'_m-1$) calculated from the data in panels A and B.

form that does not efficiently transfer excitation energy to either photosystem and fluoresces at 685 nm.

At room temperature, the fluorescence normally comes from PSII. Its yield can vary between a minimal F_0 level, at maximum photochemical quenching, and a maximum level F_m, when accumulation of reduced Q_A^- prevents photochemical activity. To estimate the effective antenna size of PSII, we measured the rise time of the light-induced fluorescence increase in the presence of DCMU, which blocks electron transfer beyond Q_A [20]. When chlorophylls were excited at an intensity that produced a half-rise time of 80 ms in control cells, the half-rise time was 28 ms in iron-starved cells, iron starvation causing a nearly 3-fold increase of the chlorophyll antenna size of PSII.

Fig. 1 shows recordings by a pulse amplitude modulated (PAM) fluorometer of light-induced Chl fluorescence yield changes in control cells (A) and iron-depleted cells (B, C) as detected by non-actinic modulated red excitation. The up and down arrows mark the beginning and end of the actinic illumination by saturating white light. The positive spikes (visible before and after the saturating illumination) indicate the fluorescence yield in the absence of photochemical quenching. The saturating illumination caused a fluorescence rise to F_m followed by a pronounced decrease due to a non-photochemical quenching in both types of cells, but the properties of the NPQ differ.

In control cells (Fig. 1A), the NPQ only quenches F_m and persists after illumination. These properties are characteristic of the quenching by PSII centers that are damaged by photo-inhibition (known as 'qI' [21]). In iron-starved cells (Fig. 1B and C), the NPQ appears much faster, quenches both F_0 and F_m, and disappears after illumination. These properties are qualitatively similar to the 'energy-dependent' NPQ of the PSII antenna in chloroplasts (known as 'qE' [21]). The difference between Fig. 1B and C is in the extent of iron starvation, 4 days and 10 days, respectively. Iron deficiency causes an increase of F_0 and F_m because the fraction of Chl associated with the low-fluorescent PSI decreases. At a later stage, F_m increases to a lesser extent than F_0 due to the increasing contribution of CP43′ fluorescence. The large NPQ reached in Fig. 1C shows that it also quenches the fluorescence of CP43′ that is not functionally connected to either photosystem. This indicates that the NPQ is associated with CP43′ and does not depend on its binding to PSII (or presumably to PSI).

The relative concentration of the non-photochemical quencher in Fig. 1A and B, quantified as $F_m/F'_m{-}1$, with F'_m denoting the quenched F_m level [8], is plotted in Fig. 1D. In control cells, NPQ steadily increased during illumination, reaching a value of 0.8 at 15 min, and did not decrease after illumination. In iron-starved cells, the NPQ rapidly reached 1.4 during the illumination, and decayed afterwards to 0.2, showing that a much smaller fraction of PSII centers was photo-inhibited than in the control cells. The decrease of the excitation rate of PSII centers by NPQ was confirmed by measurements of the

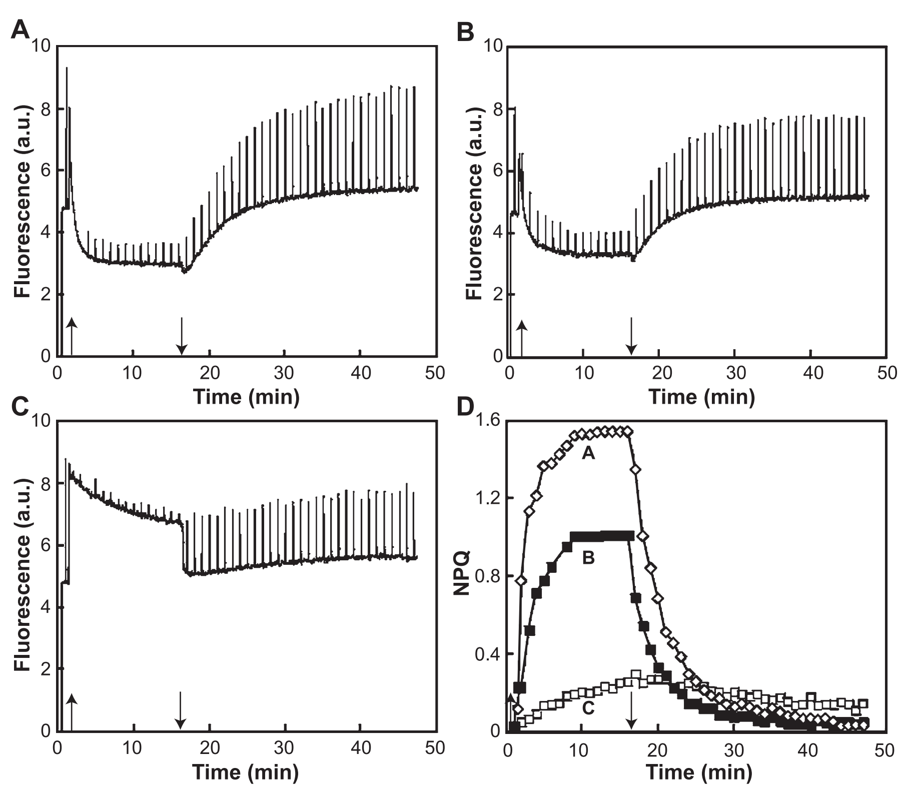

Fig. 2. Fluorescence yield changes of 4-days iron-starved cells induced by 15 min illumination by: (A) blue light (up to 600 nm, 1 mE m^{-2} s^{-1}); (B) green light (450–550 nm, 0.6 mE m^{-2} s^{-1}); and (C) orange light (>520 nm, 1 mE m^{-2} s^{-1}). (D), NPQ kinetics for panels A, B, and C.

initial slope of the saturation curve of oxygen evolution before and after NPQ induction by a continuous illumination. The slope was decreased by 40% by the presence of a NPQ of 1.5 (for F_m), in agreement with the observed quenching of F_0 [8]. We conclude that NPQ in iron-starved cells protects PSII considerably from photo-inhibition.

Fig. 2 compares the fluorescence yield changes induced in iron-starved cells by illumination in different spectral regions. The corresponding NPQ is shown in panel D. In panel A, a filter transmitting up to 600 nm was used. Panel B shows that selective excitation in the 450–550 nm region was not much less effective in inducing NPQ, although the incident light intensity was 40% lower than in panel A and clearly less saturating. Even more surprising is the result in panel C: saturating excitation at wavelengths above 520 nm, where both phycobilisomes and chlorophyll absorb, produced almost no reversible NPQ. Thus, the quenching state of CP43′ is not induced by photosynthetic electron transport. We also found no evidence for involvement of the pH gradient. The NPQ associated with CP43′ is induced only by excitation of a blue light absorbing pigment.

The initial rate of NPQ induction by blue light is proportional to light intensity; no saturation was found at intensities up to 1 mE m^{-2} s^{-1} (Fig. 3A). The decay rate

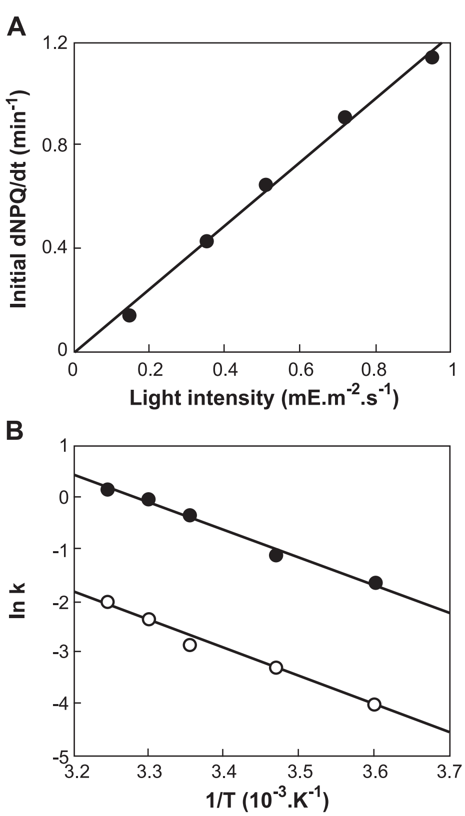

Fig. 3. Light intensity and temperature dependence of NPQ induction and decay. (A) Initial rate of the NPQ rise at the onset of illumination with blue light. (B) Arrhenius plot of the temperature dependence of this rate (solid symbols) and of the rate constant of NPQ decay after illumination (open symbols).

after illumination is mono-exponential, independent of the illumination used. Both induction and decay were strongly temperature-dependent. Fig. 3B shows that their Arrhenius plots were linear and parallel in the measured range of 5–35 °C, with an activation enthalpy of 46 kJ/mol. These findings suggest that: (i) the quenching state is formed with a temperature-dependent quantum yield from the excited state of the blue light absorbing pigment; and (ii) spontaneous reversal to the non-quenching state requires thermal activation to the same rare conformation of the local molecular environment that is required for the light-induced reaction to succeed.

Ferrous iron is an essential constituent of photosynthetic reaction centers. Qualitatively, the observed responses of *Synechocystis* to iron starvation may be explained by the need to economize on reaction center production. To compensate for their decreased abundance, the remaining reaction centers are pushed to higher activity: the CP43′ proteins added to PSI and PSII increase the absorption cross-section and hence the average excitation rate per reaction center. In photo-acclimated cells however, such an increase would lead to photo-destruction at times of saturating light intensity. In this perspective, it is not surprising that the accumulation of CP43′ coincides with the appearance of a powerful regulatory mechanism that causes dissipation of the energy absorbed by CP43′ at high light intensity.

Without CP43′, cyanobacterial PSII contains chlorophyll only in the core complex, about 35 chlorophylls/PSII. We found that CP43′ could increase the PSII chlorophyll antenna size nearly three times, so, around 65–70 chlorophylls per PSII were added. If CP43′ contains 13 chlorophylls, like its CP43 homolog, up to five CP43′ proteins can be associated with each PSII core. The same number of Pcb proteins, another homolog of CP43′, is constitutively associated with PSII in *Prochloron* and the solved structure of that complex [13] may serve as a model for that of PSII in iron-starved *Synechocystis*. Apparently, PSII cannot bind more than five CP43′ units. Additional CP43′ accumulated during prolonged iron starvation was found to be highly fluorescent, independent of the redox state of Q_A.

Blue light converts CP43′ to an efficient sink for excitation energy that substantially reduces the rate of PSII photo-inhibition in strong light. The conversion rate is proportional to light intensity, to the thermal population of a conformational state required for the conversion, and to the remaining fraction of un-converted CP43′. The quenching state is unstable and decays within tens of minutes, is independent of light intensity, but dependent on the same thermal activation. Compared to the photoprotective NPQ in chloroplasts, this simple two-state equilibrium of CP43′-associated NPQ seems relatively unsophisticated. As argued above, however, its primary function may not be to cope with rapid light intensity fluctuations, but to adjust the extra excitation rate provided by CP43′ to an already photo-acclimated system.

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