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Phycobilin/chlorophyll excitation equilibration upon carotenoid-induced non-photochemical fluorescence quenching in phycobilisomes of the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

To determine the mechanism of carotenoid-sensitized non-photochemical quenching in cyanobacteria, the kinetics of blue-light-induced quenching and fluorescence spectra were studied in the wild type and mutants of *Synechocystis* sp. PCC 6803 grown with or without iron. The blue-light-induced quenching was observed in the wild type as well as in mutants lacking PS II or IsiA confirming that neither IsiA nor PS II is required for carotenoid-triggered fluorescence quenching. Both fluorescence at 660 nm (originating from phycobilisomes) and at 681 nm (which, upon 440 nm excitation originates mostly from chlorophyll) was quenched. However, no blue-light-induced changes in the fluorescence yield were observed in the *apcE*$^-$ mutant that lacks phycobilisome attachment. The results are interpreted to indicate that interaction of the Slr1963-associated carotenoid with – presumably – allophycocyanin in the phycobilisome core is responsible for non-photochemical energy quenching, and that excitations on chlorophyll in the thylakoid equilibrate sufficiently with excitations on allophycocyanin in wild type to contribute to quenching of chlorophyll fluorescence.

Keywords: Allophycocyanin; Carotenoid; Fluorescence quenching; IsiA protein; Photosystem II; Phycobilisome

1. Introduction

Photosynthetic organisms have evolved many different strategies to be protected against excess absorbed light. When light absorption temporarily exceeds metabolic demands, cells need to harmlessly dispose of excessive excitation to prevent photodamage to the photosynthetic apparatus. In green plants, the radiation-less discharge of excessively absorbed light energy takes place via non-photochemical quenching (NPQ) monitored as a decrease in chlorophyll *a* (Chl) fluorescence of light-harvesting complex II, or LHC II [1,2]. The main component of NPQ is ΔpH-dependent quenching (qE) that is enhanced by the transformation of the carotenoid violaxanthin into zeaxanthin [3].

NPQ can be induced in cyanobacteria as well [4–6], although the organization of the cyanobacterial light-harvesting apparatus differs significantly from that of green plants. Cyanobacteria have no LHCs, and their Chls are localized in the core antennae of PS I and PS II. The light-harvesting capacity of cyanobacteria is enhanced by phycobilisomes (PBS), the extrinsic multiprotein complexes containing covalently attached bilin pigments (chromophores) arranged to facilitate migration of absorbed excitation energy to Chl in the two photosystems (reviewed by MacColl [7]). PBS has two major constitutive components: a core composed mainly of

Abbreviations: APC, allophycocyanin; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_{+BL},F_{-BL}, fluorescence yields of cells treated (+BL) or not treated (−BL) with blue actinic light; LHCII, light harvesting complex II; NPQ, non-photochemical quenching; PS II (PS I), photosystem II (photosystem I); PBS, phycobilisome; PC, phycocyanin; TE, terminal emitter

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trimeric disks of allophycocyanin (APC), and rods attached to the core with their basal part and containing stacked trimeric disks of phycocyanin (PC). PC has an absorption maximum around 610–620 nm, whereas APC can be of two functional types: the major APC is composed of α and β polypeptides with high-energy chromophores absorbing around 650–655 nm, and two polypeptides L_{CM} and α^B contain low-energy chromophores that probably serve as a bridge for energy transfer from PBS to Chl [8]. Because these low-energy chromophores emit strongly when energy transfer to Chl is restricted, they are also called terminal emitters (TE). For simplicity, in this paper the term APC is reserved exclusively for the bulk high-energy (~650 nm) pigments.

NPQ in cyanobacteria is triggered by intense blue or blue-green light [4] with the action spectrum resembling the absorption spectrum of a carotenoid dominated by a peak 480–500 nm [5,9]. A water-soluble orange carotenoid protein (OCP), with an absorption spectrum [10] that matches the NPQ action spectrum, was found to be critical for the development of blue light-induced quenching of PBS fluorescence in the cyanobacterium *Synechocystis* sp. PCC 6803 [11].

However, an alternate view [12] postulated that bright light may induce NPQ in Fe-starved cells by formation of a quenching complex between PBS and IsiA, a hydrophobic Chl-binding protein (CP43′) that is induced under stress conditions, specifically during the Fe-deficient growth, and that is similar to the CP43 protein of PS II (reviewed in Murray et al. [13]). Each IsiA polypeptide contains 16 molecules of Chl and several carotenoids [14]. IsiA can be coupled efficiently to PS I [15,16] increasing the light-harvesting capacity of the latter complex [16,17]. IsiA has been suggested to be involved in photoprotection [14,18–20] by quenching Chl excited states [6,14,21]. However, carotenoid-triggered quenching of PBS fluorescence was observed even in the *isi*A$^-$ mutant of *Synechocystis* sp. PCC6803 grown on under non-stress conditions [12,22].

In the present work, we studied the effects of strong blue light on the fluorescence emission spectra of several *Synechocystis* sp. PCC6803 strains grown with or without iron. We demonstrate that blue-light-induced NPQ in this cyanobacterium quenches fluorescence of both PBS and Chl, and that the quenching is enhanced in iron-starved cells. These phenomena can be explained by enhanced energy dissipation from APC of the PBS core, which is in excitation contact with Chl in the thylakoid.

2. Materials and methods

2.1. Strains and growth conditions

The wild type of *Synechocystis* sp. PCC 6803 was obtained from the Culture Collection of the Department of Genetics of the Moscow State University. The PS II-less strain was created by inactivating *psb*B [23] and *psb*DIC genes using DNA isolated from the corresponding single-gene deletion mutants. Therefore, this strain is unable to synthesize CP47 and CP43. The deletion of *psb*DIC was originally introduced in *Synechocystis* sp. PCC 6803 by replacing a 2818-bp *XmnI/SfiI* fragment, which covered the entire *psb*DIC coding region from 212 bp upstream of *psb*DI to 172 bp downstream of *psb*C, with a 1.7 kb erythromycin resistance cassette [S. Ermakova-Gerdes and W. Vermaas, unpublished]. The deletion of *isiA* was introduced by replacing a 448-bp *Apa*I–*Hin*cII portion of the *isi*A (nucleotides 338–785 downstream of the start codon) with a 1.3-kbp kanamycin resistance cassette from a pUC4K plasmid [Q. He and W. Vermaas, unpublished]. The wild-type and IsiA-less strains were grown in BG-11 medium [24] in 100 ml flasks at 30 °C under continuous white fluorescence light at a photon density of 40 μE m^{-2} s^{-1}. The PS II-less strain was grown at a photon density of 12 μE m^{-2} s^{-1}. The medium used to grow the mutants was routinely supplemented with 100 μg ml^{-1} kanamycin (*isi*A$^-$ cells) or with 20 μg ml^{-1} streptomycin, 20 μg ml^{-1} erythromycin, and 10 mM glucose for PS II-less cells. However, in separate experiments it was verified that the presence of the antibiotics in the growth media had no effect on fluorescence parameters. To grow iron-deficient cells, the BG-11 medium lacked ferric ammonium citrate and glassware was acid-washed before use. All experiments were performed with 4-day cultures that were in the logarithmic growth phase.

2.2. Spectral measurements

Room-temperature absorption spectra were recorded using a Beckman 650 UV/VIS spectrophotometer.

The kinetics of NPQ fluorescence quenching was monitored with a PAM-101 fluorometer (Walz, Germany). In these experiments, a *Synechocystis* suspension (1 mm pathlength) contained in a home-built mirrored cuvette at 288 K was excited through fiber optics with a red modulated light (650 nm) and the fluorescence was detected at $\lambda > 700$ nm. DCMU (20 μM) was added to the cells; at the modulated light intensity used all PS II centers were in the closed state during the measurements. To induce NPQ, cells were additionally exposed to blue (500 nm, bandwidth 50 nm, intensity of 1000 μE m^{-2} s^{-1}) or red (650–675 nm bandwidth 40 nm, intensity of 1300 μE m^{-2} s^{-1}) actinic light provided by a KL-1500 illuminator (Schott, Mainz, Germany) equipped with appropriate glass filters. Samples for PAM measurements containing about 10 μg ml^{-1} Chl were normalized to PBS absorption at 625 nm.

Fluorescence spectra were recorded with a Shimadzu RF-5301PC instrument. Cell suspensions (2 μg ml^{-1} Chl and 20 μM DCMU) were placed in a cuvette with a 3 or 1 mm optical path to measure fluorescence at 288 K or 77 K, respectively. The measurements were carried out upon 440 or 580 nm excitation; the bandwidths of excitation and emission monochromators were set at 3 nm. To determine changes induced by blue light, 288 K fluorescence spectra of every sample were recorded twice: before and right after a 4-min exposure to quenching light (500 nm; photon flux density about 1100 μE m^{-2} s^{-1}) provided by the excitation beam of the spectrofluorometer, in which the bandwidth of excitation monochromator was temporarily increased to 15 nm. 288 K emission spectra upon 440 nm excitation were normalized to Chl content. 288 K fluorescence spectra upon 580 nm excitation were normalized to PC absorption at 625 nm. 77 K spectra except for fluorescence quenching spectra were normalized to the emission band at 725 nm (440 nm excitation) and to the emission band at 650 nm (580 nm excitation).

Changes in 77 K fluorescence emission spectra upon exposure of *Synechocystis* cells to blue quenching light were recorded using a Fluorolog 2 instrument (Spex Industries, NJ, USA); the optical parameters and cell concentrations were identical to those used for the Shimadzu spectrofluorometer. Cells were placed in glass tubes (4 mm diameter) that were dipped in liquid nitrogen before or after the blue light treatment. Dichlorofluorescein diacetate pre-treated with H_2O_2 was added to the cell suspensions to a final concentration of 1 μM immediately before the samples were frozen to facilitate normalization of the spectra. The 77 K fluorescence was measured in each sample upon 440, 480 and 580 nm excitations and the fluorescence signals induced by 440 and 580 nm light were subsequently normalized to the intensity of fluorescein emission integrated over the 500–580 nm spectral range (excitation at 480 nm).

3. Results

3.1. Spectral characteristics of mutants

Fig. 1A–D compares fluorescence spectra of wild-type, IsiA-less and PS II-less *Synechocystis* cells grown in BG-11

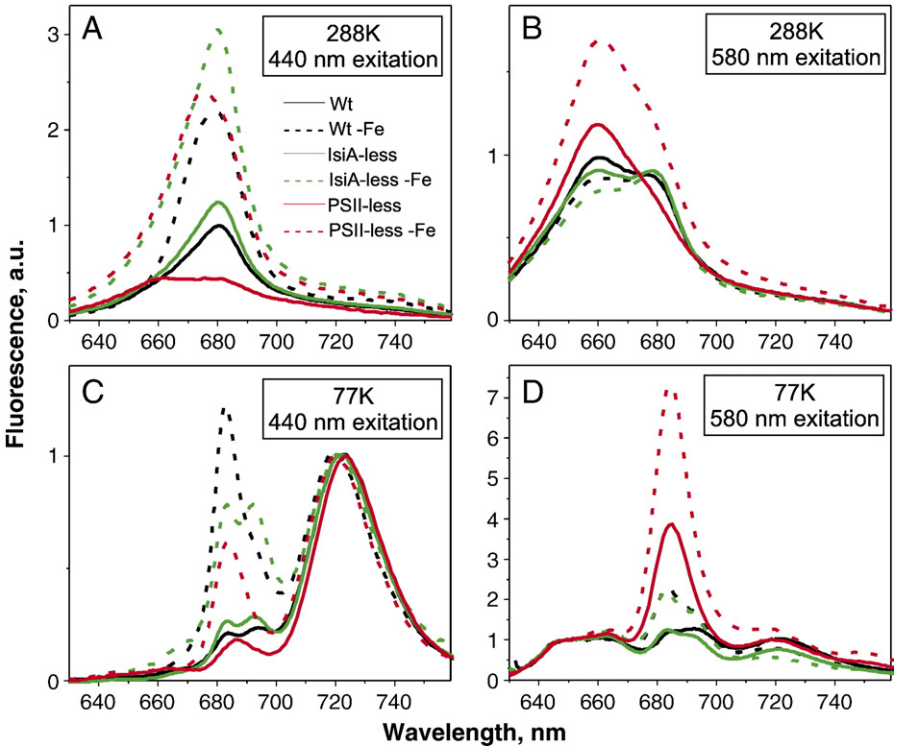

Fig. 1. Fluorescence spectra of wild type, IsiA-less and PS II-less strains of the cyanobacterium *Synechocystis* sp. PCC 6803 grown under iron-replete (solid lines) or iron-deficient (dash lines) conditions measured at 288 (A, B) or 77 K (C, D). 77 K spectra were normalized to the emission band at 725 nm (440 nm excitation) and to the emission band at 650 nm (580 nm excitation); 288 K emission spectra are normalized to Chl content (440 nm excitation) or to PBS content (580 nm excitation); the intensity of the fluorescence maximum of the wild-type strain grown in the presence of iron was taken as unity.

medium with and without ferric ammonium citrate. To measure fluorescence, cell samples were excited with 440 nm light that is absorbed preferentially by Chl and to a much lesser extent by phycobilins, or with 580 nm light absorbed almost exclusively by phycobilins. The spectral properties of the wild type and the IsiA-less mutant grown under iron-replete conditions were similar indicating that in the presence of sufficient iron inactivation of the *isi*A gene had little effect on the photosynthetic apparatus of *Synechocystis* cells. As expected, 440 nm excitation of wild type and IsiA-less cells at 77 K induced PS II (683 and 693 nm peaks) and PS I (721 nm peak) fluorescence (Fig. 1C). Upon 580 nm excitation at 77 K (Fig. 1D), emission peaks or shoulders observed in these two strains were at 650 nm (PC), 665 nm (APC), 684 nm (TE and some PS II Chl), 693 nm (PS II Chl), and 721 nm (PS I Chl). In the 77 K fluorescence emission spectrum of the PS II-less mutant (440 nm excitation) the 693 nm band was absent, consistent with its assignment to PS II. In this strain, upon selective phycobilin excitation the 684 nm fluorescence peak was much more prominent, relative to the 721 nm fluorescence peak (Fig. 1D), which correlates with the observation that PBS in PS II-less cells energetically are coupled only partially to PS I [25,26]. Consistent with this interpretation, PBS fluorescence that peaks at 660 nm dominated in the 288 K spectrum as well. In PS II-less cells, Chl fluorescence at 288 K was much lower than in the wild-type or IsiA-less strains due to the substantially shorter lifetime of the excited state of PS I as compared to that of PS II [27].

Iron deprivation of cyanobacteria generally results in a shift of the red Chl absorption peak to shorter wavelengths due to accumulation of the Chl-binding IsiA protein [28,29]. Indeed, second-derivative absorption spectra (Fig. 2) illustrate the shift of the Chl absorption maximum from 683 to 674 nm in the wild type and PS II-less but not in IsiA-less cells of *Synechocystis* that were grown without iron. IsiA complexes can form rings around PS I [15,16,20] that are organized mostly as trimers (reviewed in [30]) but undergo some monomerization under iron deficiency [31]. Structural and spectroscopic studies indicate that IsiA interacts equally well with monomeric or trimeric PS I [29]. There are also indications that IsiA may interact with PS II [19] and a significant IsiA pool appears to be free in the membrane [20]. Accumulation of significant amounts of free IsiA complexes that cannot transfer energy to photosystems was evident from the 675 nm Chl peak in the 288 K fluorescence spectra of iron-starved PS II-less cells (Fig. 1A). 77 K excitation of these iron-starved PS II-less cells at 440 nm yielded a sharp fluorescence band at 683 nm (Fig. 1C), corresponding to the 77 K fluorescence maximum of isolated IsiA aggregates [14]. The fact that the 288 K fluorescence spectrum of iron-deficient PS II-less cells recorded upon PBS excitation had only a small shoulder around 675–680 nm being essentially identical to the spectrum of iron-replete PS II-less *Synechocystis* (Fig. 1B) indicates that little energy transfer between PBS and IsiA that is uncoupled from photosystems occurred.

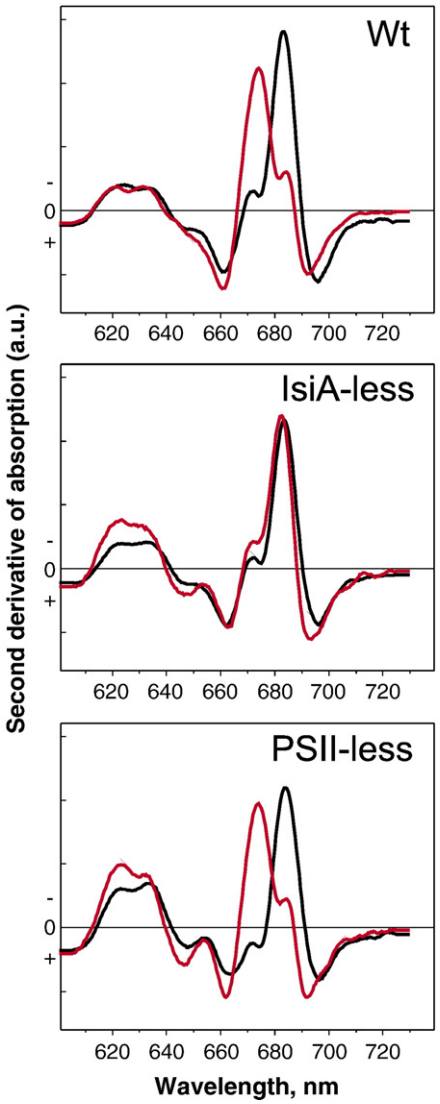

Fig. 2. Second derivatives of the absorption spectra grown with (black) or without (red) iron measured for the wild-type, IsiA-less and PS II-less strains of the cyanobacterium *Synechocystis* sp. PCC 6803.

As expected, iron deficiency also caused an increase in the PS II/PS I ratio, which was apparent particularly from comparing the 77 K fluorescence spectra upon Chl excitation of the IsiA-less mutant: upon iron limitation, the intensity of the 725 nm band was decreased relative to that of the 685/695 nm emission (Fig. 1C). It should be also noted that in all three *Synechocystis* strains used in our experiments iron starvation caused an increase in fluorescence from the TE (685 nm) elicited by 580 nm excitation (Fig. 1D). However, the intensity of PS I fluorescence (720 nm) in iron-starved and iron-replete cells was comparable, relative to phycobilin emission 650 nm, suggesting that the overall efficiency of energy transfer from phycobilin pigments to Chl was comparable under the two conditions. Therefore, the increased TE fluorescence intensity under iron-starved conditions may be due to a small population of disconnected (and thereby highly fluorescent) PBS formed upon freezing. In line with this argument, no major changes in APC (660 nm) fluorescence intensities were apparent upon selective PBS excitation (Fig. 1B) except in the PS II-less strain. As will be presented in the next section, in the PS II-less strain NPQ is much higher under iron-limited conditions, with the final fluorescence intensity (after quenching) comparable to that of the other strains. Therefore, regardless of the reason for increased PBS-induced fluorescence in the PS II-less strain under iron-limited conditions, it may be relevant for understanding NPQ in this system.

3.2. Kinetics of fluorescence quenching

Strong fluorescence quenching was induced by blue-green actinic light (1000 μE m^{-2} s^{-1}) in all three strains grown with or without iron (Fig. 3). For the experiments presented in Fig. 3, DCMU was added prior to exposure to strong light in order to prevent contribution of photochemical quenching or state transitions to changes in fluorescence yield. Fluorescence quenching was not observed upon illumination of cyanobacteria with red actinic light of the same intensity (1000 μE m^{-2} s^{-1}), indicating that the changes in fluorescence yield in the time range of minutes cannot be attributed to PS II photodamage. The kinetics of blue-light-induced fluorescence quenching were similar in the wild type, PS II-less, and IsiA-less strains grown with (half life-time ~22 s) or without (~18 s) iron, suggesting that the same mechanism is responsible for the observed changes in fluorescence intensity. The rate of fluorescence recovery to the non-quenched state was slower than published before [4–6] due to a lower temperature (288 K) in the current study.

The degree of quenching measured with the PAM fluorometer was in the range of 40 to 55% of the initial fluorescence value in the wild-type, IsiA or PS II-less strains grown in iron-sufficient media, as well as in the wild-type or IsiA-less strains grown under iron-limited conditions (Fig. 3). However, the fluorescence quenching was substantially larger (up to 80%) in the strain lacking PS II if grown under iron-limited conditions. Note, however, that under these conditions the overall fluorescence intensity in the non-quenched state was significantly higher when the PS II-deficient mutant was grown without iron (Fig. 1).

3.3. Fluorescence quenching spectra

Phycobilin and Chl molecules both emit fluorescence in the spectral region detected by the PAM-101 fluorometer. To determine whether the blue-light-induced quenching can be associated with specific pigments, 288 K fluorescence emission spectra before and after the 4-min light treatment were recorded. The corresponding difference spectra were normalized to the intensity of the fluorescence emission in the non-quenched state integrated over the 600–750 nm wavelength range and the results are presented in Fig. 4. Fig. 4C shows the blue-light-induced changes in the total yield of fluorescence (NPQ, %) calculated as $100\times(\int F_{-BL}-\int F_{+BL})/\int F_{-BL}$, where $\int F_{-BL}$ and $\int F_{+BL}$ indicate fluorescence emissions in the non-quenched and quenched states, respectively, integrated over the 600–750 nm wavelength range. In all cultures the quenching was more

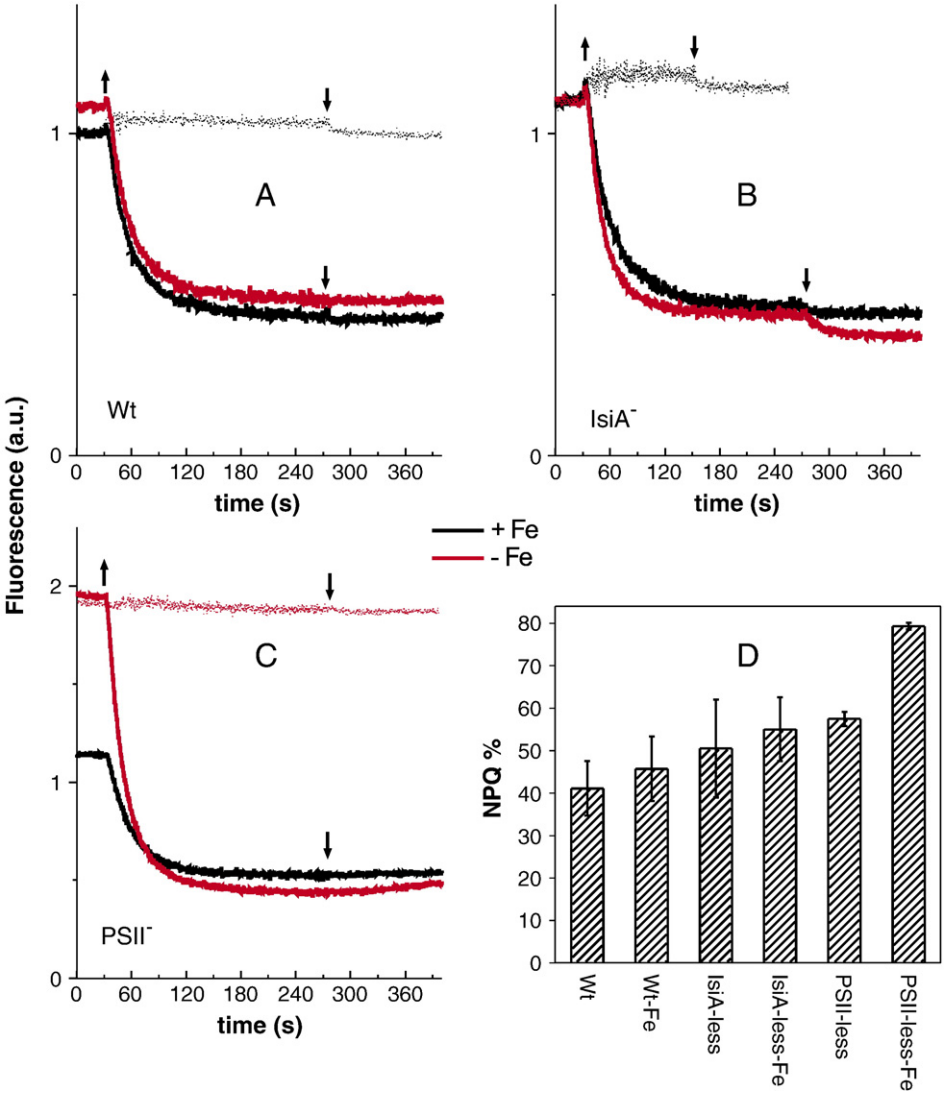

Fig. 3. Time course (A–C) and degree (D) of fluorescence quenching in the wild-type (A), IsiA-less (B), and PS II-less (C) strains of the cyanobacterium *Synechocystis* sp. PCC 6803 grown under iron-replete (black) or iron-deficient (red) conditions. The measurements were performed with a PAM-101 fluorometer at 288 K with an actinic light intensity (480–500 nm) of 1000 μE m^{-2} s^{-1}. No fluorescence quenching was observed under red actinic light (650–680 nm) at the same intensity (shown as weak lines). Degree of quenching (NPQ %) was calculated as $(1-F_{+BL}/F_{-BL})\times 100$.

pronounced when fluorescence was measured upon the cell excitation at 580 nm rather that at 440 nm, with the most significant changes in NPQ observed in the PS II-less strain grown without iron (Fig. 4).

Upon excitation of PBS at 580 nm the fluorescence quenching spectra in the wild-type and IsiA-less cells showed minima around 660 and 680 nm, likely associated with a decreased fluorescence emission from APC in the PBS core and from Chl and/or the TE, respectively, after *Synechocystis* cells had been exposed to blue light (Fig. 4A). The quenching spectra of the iron-starved wild type and *IsiA-less* strains were similar to those in iron-replete cultures, although the decrease in fluorescence around 680 nm in iron-replete cells was less pronounced. Upon predominant excitation of Chl (440 nm), the quenching spectra of the two strains showed a well-resolved minimum at 679 nm and a poorly-resolved shoulder in the 650–670 nm spectral region (Fig. 4B). Analysis of the second derivatives of these difference spectra (not shown) indicated that the 650–670 nm shoulder reflected a decrease in fluorescence centered around 660 nm and therefore is likely to be associated with accelerated de-excitation of APC, whereas quenching at 679 nm is attributed to quenching of Chl and/or TE emission.

The fluorescence difference spectrum of NPQ quenching in the PS II-less strain showed maximal amplitude at ~660 nm, with a poorly resolved shoulder around 680 nm (or 677 nm in second derivatives), regardless of the presence of iron in the growth media and the excitation wavelength used to record the emission spectra (Fig. 4). This indicates that in this mutant most of fluorescence quenching was associated with APC rather than with Chl or the TE. In agreement with [11], no blue-light-induced changes in the fluorescence yield were observed in the *apc*E⁻ mutant of *Synechocystis* (Fig. 4), which is impaired in assembly of the PBS core complex [32].

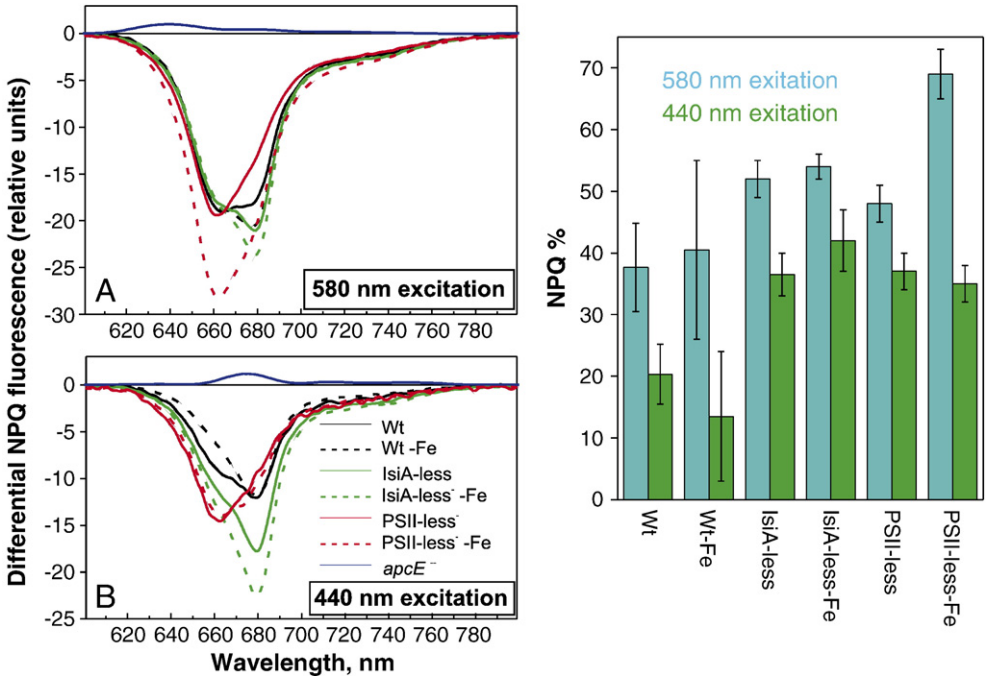

Fig. 4. Spectra and degree (diagram) of fluorescence quenching in the wild-type, IsiA-less, PS II-less, and apcE- strains of the cyanobacterium *Synechocystis* sp. PCC 6803 grown under iron-replete (solid) or under iron-deficient (dash) conditions measured upon PBS (A) or Chl (B) excitation. The curves were obtained by subtracting fluorescence spectra measured before light exposure from those measured after a 4-min blue-light treatment. Each difference spectrum was normalized to the value of the integrated emission of the same sample before illumination. NPQ values (%) were calculated as $100\times(\int F_{-BL}-\int F_{+BL})/\int F_{-BL}$, where $\int F_{-BL}$ and $\int F_{+BL}$ indicate fluorescence emissions in the non-quenched and quenched states, respectively, integrated over the 600–750 nm wavelength range. All fluorescence measurements were performed at 288 K.

In contrast to spectra measured at 288 K, very little difference in the fluorescence emission spectra of dark-adapted and blue-light-treated wild type cells was found when fluorescence was recorded at 77 K upon Chl excitation at 440 nm (Fig. 5). For example, the intensity of Chl fluorescence emission integrated over the 670–760 nm spectral range in the blue-light-treated samples was 97±5% of the dark-adapted control (mean±s.d. of five separate measurements using two different cultures), which is in line with earlier observations [11,21]. However, blue-light-induced 77 K fluorescence quenching was significant upon excitation at 580 nm (Fig 5). In average, the intensity of 77 K PBS fluorescence emission integrated over the 630–760 nm range in the blue- light-quenched samples comprised only 61±14% of the untreated control. The difference spectrum of quenching showed a main peak at 685 nm with smaller peaks at 665 and 725 nm, suggesting a significant decrease in the fluorescence associated with the bulk of APC pigments (665 nm) and with the TE of PBS (685 nm), resulting in less excitation to be transferred to the photosystems (685 and 725 nm, and a shoulder at 695 nm).

4. Discussion

The comprehensive comparison of fluorescence spectra in several *Synechocystis* mutants impaired in the assembly of functional pigment–protein complexes and grown under iron-replete or iron-deficient medium presents new insights into energy transfer and energy quenching in cyanobacteria. The results show that depending on experimental conditions, Chl and/or phycobilin fluorescence can be quenched after exposure to strong blue light. As illustrated by the scheme presented in Fig. 6, the observed changes in the fluorescence emission accompanying NPQ in cyanobacteria are best explained by enhanced energy dissipation from APC [9,11]. Short-wavelength forms of APC, rather than the TE, are responsible for the blue-light-induced NPQ as evidenced by several lines of evidence.

First, upon excitation at 580 nm rather strong fluorescence quenching at 660 nm (marked as F660 in Fig. 6) was observed in all strains having assembled PBS (Figs. 4 and 5). In contrast, the quenching was absent in the *apc*E$^-$ strain, which was deficient in PBS core components but had intact PS I, PS II, and PBS rods [32]. Second, excitation of the PS II-less mutant with 580 nm light or even with 440 nm light, absorbed predominantly by Chl, caused a selective decrease in fluorescence at 660 nm indicative of quenching of PBS fluorescence (Fig. 4). In agreement with results presented in [11], fluorescence quenching was not observed in wild-type cells when measurements were performed upon Chl excitation at 77 K (Fig. 5). Finally, the 77 K spectra of blue-light-treated wild type cells (Fig. 5) showed a decreased fluorescence yield of 665 and 685 nm components (due to APC and TE/Chl, respectively), whereas fluorescence of PC (640–650 nm emission region) remained unquenched.

Changes in the 77 K spectrum of PBS fluorescence upon blue-light-induced quenching suggested that quenching originated mainly from short-wavelength phycobilin pigments

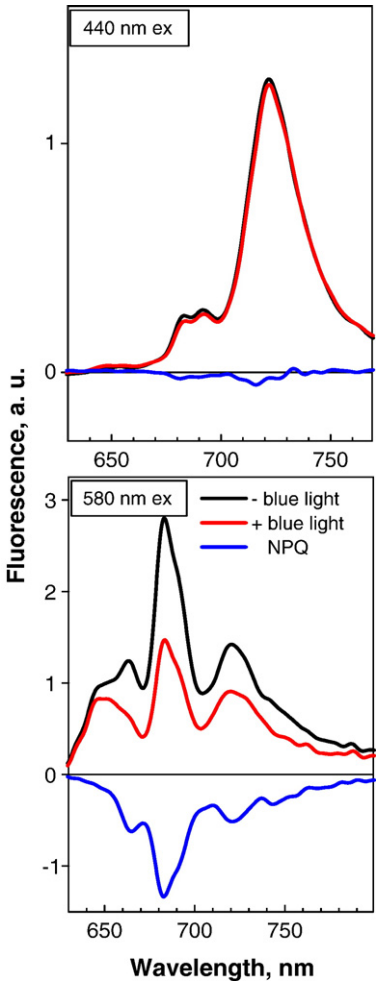

Fig. 5. Fluorescence quenching of wild type cells. 77 K fluorescence spectra were measured before and after blue-light exposure for 4 min at 288 K; the NPQ spectrum presents the difference between quenched (red) and unquenched (black) spectra. Spectra were normalized as described in Materials and methods. The results of a typical experiment are shown.

associated with the α and β subunits of APC. Indeed, upon excitation of PBS, the 580 nm light was absorbed mostly by PC and APC, and excitation energy was transferred to TE. Therefore, the increased quenching of excitation energy by APC resulted in decreased fluorescence of both APC and TE, which is consistent with the spectrum shown in Fig. 5. We interpret fluorescence quenching to originate from APC rather than from the TE or Chl as the fluorescence yield of APC was decreased, and uphill energy transfer from TE to APC would be very much retarded at low temperature. Also, if TE would be involved in quenching the 77 K fluorescence yield of PS II would be decreased since most of the PS II pigments are essentially isoenergetic with TE. Consequently, the fact that Chl fluorescence is not quenched at low temperature is consistent with a blue-light-induced quenching mechanism that accelerates de-excitation of APC rather than TE or Chl pigments. The localization of quenching to shorter wavelength APC core components was recently observed by Scott et al. [9] in the decay associated fluorescence spectra of blue light quenched cyanobacteria.

Therefore, APC quenching decreases the fluorescence yield of Chl at room temperature (see Figs. 4 and 6). Upon excitation of PBS at 580 nm the energy absorbed by PC migrates to APC in about 20–30 ps, whereas energy transfer from APC to Chl of PS II takes from 80–90 [26] to 150–170 ps [27,33]. Taking into account the nearly 20 nm difference in the position of Qy absorption band of Chl of PS II and APC, the rate of reverse energy transfer from PS II to APC at room temperature is expected to be in the range of 800–1700 ps (~10-fold slower than energy migration from APC to PS II). Two major components of Chl fluorescence decay with the lifetimes of 40–70 ps and 300–410 ps can be associated with the open PS II centers. Upon closing the PS II reaction centers lifetimes of these components increase to 310–640 and 1200–1500 ps, respectively, with the slowest component becoming the major contributor to the overall Chl fluorescence yield [26,27,33]. In our experiments, fluorescence was measured upon excitation of samples at a relatively high light intensity in the presence of DCMU when nearly all PS II centers were in the closed state with the average rate of energy dissipation comparable to the predicted rate of energy transfer from PS II to APC (800–1700 ps, see above). Consequently, excitation of closed PS II should be accompanied by migration of a portion of excitation energy to the PBS core. If APC is converted to the quenched state by blue-light treatment, this energy transfer from Chl to APC is anticipated to cause a noticeable decrease in Chl fluorescence (F681 in Fig. 6). However, if fluorescence spectra were recorded under conditions when majority of PS II centers remained open, the lifetime of excitations in PS II would be too short for the excitation energy to have enough time to migrate to PBS. Therefore, no decrease in the overall fluorescence would be expected in this case. In fact, the absence of Chl fluorescence quenching at room temperature upon direct Chl excitation was reported by Wilson and co-authors [11], suggesting that their measurements were done under conditions of open PS II centers.

The absence of Chl fluorescence quenching in the PS II-less strain could be explained by functional decoupling of PBS from PS I. Even if some PBS were connected to PS I, blue light treatment would not quench fluorescence of PS I-associated Chl because the lifetime of the excited state of cyanobacterial PS I (20 to 40 ps) [26,27] is too short to allow for energy migration from PS I to PBS (Fig. 6).

The same mechanism of NPQ involving accelerated energy dissipation in the PBS core is likely to be responsible for the blue-light-stimulated decrease in the fluorescence yield of iron-starved *Synechocystis* cells, even though it was hypothesized that binding of IsiA to free PBS facilitated energy dissipation from the latter complex due to much shorter lifetime of excitations in IsiA aggregates than in isolated PBS (~210 ps versus 1.4 ns) [12]. Taking into account that the blue-light-induced fluorescence quenching in PBS core can compete effectively with the energy transfer from PBS to PS II occurring on the time-scale of 80 to 170 ps [26,27,33], coupling of IsiA and PBS should not substantially decrease the lifetime of excitations in PBS. Moreover, as the energy difference between PBS and IsiA corresponds to about 15 nm

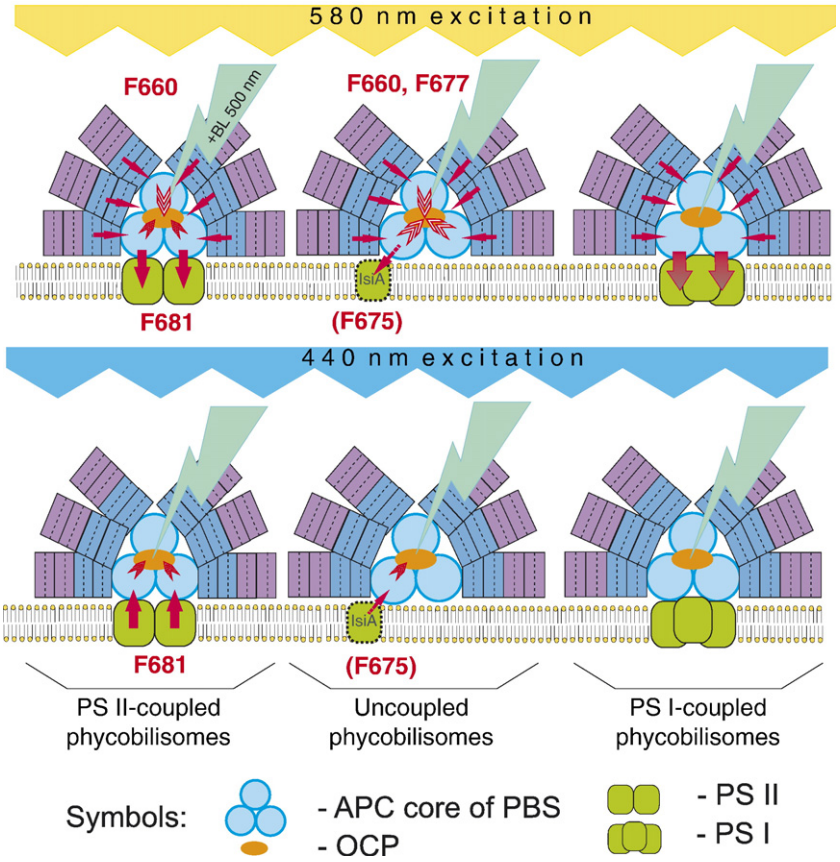

Fig. 6. Scheme describing the origin of OCP-induced non-photochemical quenching of APC and Chl fluorescence in cells of *Synechocystis* sp. PCC 6803. F660 reflects quenching of the APC core of coupled PBS, F660/F677 reflects the quenching of the APC core/TE of uncoupled PBS in the PSII-less mutant, and F681 quenching is attributed to Chl of PS II; the IsiA complex formed at iron starvation may be responsible for F675 quenching. Fluorescence quenching data are taken from the spectra in Fig. 4.

in terms of their maximum absorption in the red region of the spectrum, and the rate of reverse energy transfer from IsiA to PBS would be expected to be only 5 times slower than the excitation migration rate from PBS to IsiA. Consequently, excitations residing on IsiA would have a reasonable chance to be transferred to APC and finally get quenched in the PBS (F675 in Fig. 6).

The light-stimulated NPQ in *Synechocystis* has been shown to be sensitized by a carotenoid [5,9], possibly 3′-hydroxyechinenone [33] associated with the water-soluble carotenoid-binding protein (OCP) [11,34,35]. The OCP protein is likely to play a central role in the quenching mechanism by functioning as a light receptor, transducer of the quenching or as the quencher itself [11]. Since the OCP is localized mostly in the interthylakoid region [11] OCP-bound carotenoids may be directly responsible for energy quenching through interaction with phycocyanobilin pigment(s) of the PBS core. In fact, the solvent-exposed hydroxy-terminus of the carotenoid is localized close to the protein surface [35], potentially allowing it to be in close proximity to APC chromophores (Fig. 6). Although the molecular details of NPQ mechanisms in cyanobacteria await further studies, it is clear that this process cannot only reduce the amount of energy delivered from PBS to Chl-protein complexes in the thylakoid membranes, but also effectively quench Chl excitations localized in closed PS II centres via somewhat uphill energy transfer to PBS, thereby providing protection of this vulnerable complex from photodamage by excessive irradiation.

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