Light-Induced Energy Dissipation in Iron-Starved Cyanobacteria: Roles of OCP and IsiA Proteins

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In response to iron deficiency, cyanobacteria synthesize the iron stress–induced chlorophyll binding protein IsiA. This protein protects cyanobacterial cells against iron stress. It has been proposed that the protective role of IsiA is related to a blue light–induced nonphotochemical fluorescence quenching (NPQ) mechanism. In iron-replete cyanobacterial cell cultures, strong blue light is known to induce a mechanism that dissipates excess absorbed energy in the phycobilisome, the extramembranous antenna of cyanobacteria. In this photoprotective mechanism, the soluble Orange Carotenoid Protein (OCP) plays an essential role. Here, we demonstrate that in iron-starved cells, blue light is unable to quench fluorescence in the absence of the phycobilisomes or the OCP. By contrast, the absence of IsiA does not affect the induction of fluorescence quenching or its recovery. We conclude that in cyanobacteria grown under iron starvation conditions, the blue light–induced nonphotochemical quenching mechanism involves the phycobilisome OCP–related energy dissipation mechanism and not IsiA. IsiA, however, does seem to protect the cells from the stress generated by iron starvation, initially by increasing the size of the photosystem I antenna. Subsequently, the IsiA converts the excess energy absorbed by the phycobilisomes into heat through a mechanism different from the dynamic and reversible light-induced NPQ processes.

INTRODUCTION

Excess light can be lethal for photosynthetic organisms because harmful reactive oxygen species are generated in the photochemical reaction centers when energy absorption exceeds the rate of carbon fixation. To survive, photosynthetic organisms have evolved several protective processes. One such mechanism is the dissipation of the excess absorbed energy as heat in the light-collecting pigment/protein complexes, the so-called antenna. In plants, this process involves the chlorophyll-containing light-harvesting complex (LHCII) of photosystem II (PSII) and is triggered by acidification of the thylakoid lumen under saturating light conditions (reviewed in Demmig-Adams, 1990; Horton et al., 1996; Niyogi, 1999; Müller et al., 2001). A drop in the thylakoid lumen pH activates the formation of the carotenoid zeaxanthin from violaxanthin as part of the xanthophyll cycle (Yamamoto, 1973; Gilmore and Yamamoto, 1993) and induces the protonation of PsbS, a PSII subunit that belongs to the LHCII superfamily (Li et al., 2000, 2004). This process also involves conformational changes in LHCII, modifying the interaction between chlorophylls and carotenoids (Ruban et al., 1992; Pascal et al., 2005). Thermal energy dissipation is accompanied by a decrease of PSII-related fluorescence emission, known as high-energy quenching (qE), one of the nonphotochemical quenching (NPQ) processes. The qE is a dynamic, rapidly reversible process that is induced seconds after the plant is exposed to high light intensities.

Several recent studies have shown that cyanobacteria, which do not have the integral membrane chlorophyll-containing LHCII, also use a light-induced antenna-related NPQ mechanism to decrease the amount of energy funneled to the PSII reaction center (El Bissati et al., 2000; Rakhimberdieva et al., 2004; Scott et al., 2006; Wilson et al., 2006). In cyanobacteria, light is captured by a membrane extrinsic complex, the phycobilisome, which is attached to the outer surface of thylakoid membranes. These large complexes consist of phycobiliproteins with covalently bound bilin pigments and linker peptides that are required for the organization of the phycobilisomes (reviewed in MacColl, 1998; Adir, 2005). Phycobilisomes are composed of a core from which rods (usually six) radiate. The major core protein is allophycocyanin (APC), while the rods contain phycocyanin (PC) and, in some species, phycoerythrin or phycoerythrocyanin (in the distal end of the rod). The phycobilisomes are bound to the thylakoids via the core membrane linker protein Lcm, which also serves as the terminal energy acceptor. Harvested light energy is transferred from Lcm to the chlorophylls of PSII and photosystem I (PSII) (Mullineaux, 1992; Rakhimberdieva et al., 2001).

Results revealing the existence of a blue light–induced NPQ mechanism proposed to be associated with the phycobilisomes were first described in 2000 (El Bissati et al., 2000). Subsequently, spectral and kinetics data were presented suggesting that blue light–activated carotenoids induce quenching of
phycobilisome fluorescence (Rakhimberdieva et al., 2004). Wilson et al. (2006) demonstrated that a soluble carotenoid binding protein, the Orange Carotenoid Protein (OCP), is specifically involved in a phycobilisome-related NPQ that appears to be associated with a photoprotective energy dissipation mechanism. OCP, a 35-kD protein that contains a single non-covalently bound carotenoid, is encoded by the slr1963 open reading frame in Synechocystis PCC 6803 (Holt and Krogmann, 1981; Wu and Krogmann, 1997; for review, see Kerfeld, 2004a, 2004b). Highly conserved homologs of OCP are found in the genomes of all cyanobacteria, with the exception of the Prochlorococcus strains, for which genomic data are available (Kerfeld, 2004a, 2004b).

In the absence of OCP, the NPQ induced by strong white or blue-green light in Synechocystis PCC 6803 cells is completely inhibited, and the cells are more sensitive to high light intensities (Wilson et al., 2006). The observation that the effective antenna size was smaller in the cells in the quenched state strongly supports the hypothesis that the OCP-related mechanism dissipates the excess absorbed energy, thereby decreasing the amount of energy arriving at the photochemical centers. The OCP phycobilisome–associated NPQ is not dependent on the presence of a transthylakoid ΔpH, on the excitation pressure on PSII, or on changes in the redox state of the plastoquinone pool (El Bissati et al., 2000; Scott et al., 2006; Wilson et al., 2006). Instead, OCP seems to act as a photoreceptor that responds to blue-green light and induces energy dissipation (and fluorescence quenching) through interaction with the phycobilisome core.

Under iron starvation conditions, blue light causes a large reversible quenching of Fo and Fm levels (Cadoret et al., 2004; Bailey et al., 2005; Joshua et al., 2005). It was proposed that the iron stress–induced protein IsiA was essential in this NPQ process. Two mechanisms were proposed: (1) blue light converts the IsiA protein from one form that is efficient in harvesting light energy for photosynthesis into another form that converts excess energy into heat (Cadoret et al., 2004); and (2) strong light induces a change in IsiA, increasing the affinity of IsiA for the phycobilisomes and diminishing the high fluorescence of free phycobilisomes (Joshua et al., 2005).

The expression of the IsiA protein, which belongs to the core complex family of chlorophyll binding proteins, is induced by iron starvation (Laudenbach and Straus, 1988; Burnap et al., 1993) and other stress conditions (such as salt stress, oxidative stress, and high-light stress) (Jeejeebhoy et al., 2003; Yousef et al., 2003; Havaux et al., 2005). IsiA encircles the PSI reaction center, forming complexes consisting of a trimeric PSI and 18 IsiA molecules (Bibby et al., 2001; Boekema et al., 2001). Larger

Figure 1. Changes in Absorption Spectra and Chlorophyll Content Induced by Iron Starvation in Wild-Type, ΔOCP, and ΔIsiA Cells.

(A) and (B) Absorption spectra of wild-type (A) and ΔOCP (B) cells grown in iron-containing medium (solid line) or in iron-depleted medium for 12 (small dashed line), 14 (dotted line), or 20 (large dashed line) d.

(C) Absorption spectra of ΔIsiA cells grown in iron-containing medium (solid line) or in iron-free medium for 7 (large dashed line), 12 (small dashed line), or 14 (dotted line) d. The spectra were normalized at OD800.

(D) Decrease of chlorophyll content in iron-starved wild-type (circles), ΔOCP (triangles), and ΔIsiA (squares) cells. The results are the average of seven independent experiments. Error bars show the maximum and minimum chlorophyll/OD800 values for each point. 100% of chlorophyll/OD800 = 7.5, corresponding to ~5.8 μg chlorophyll/mL for a culture at OD800 = 0.78.
amounts of IsiA are bound to PSI during prolonged iron starvation (Yeremenko et al., 2004). IsiA increases the absorptive cross section of PSI, acting as an additional and efficient LHC for PSI (Andrizhiyevskaya et al., 2002; Melkozernov et al., 2003). IsiA aggregates, forming empty multimeric rings (without PSI), also accumulate and are very abundant in long-term iron-depleted cells (Yeremenko et al., 2004). These IsiA aggregates, in vitro, are in a strongly quenched state, suggesting that they are responsible for thermal dissipation of absorbed energy (Ihalainen et al., 2005). In cells grown under high light conditions, IsiA is also synthesized to protect the cyanobacterial cells from photodestruction (Havaux et al., 2005).

In addition to IsiA, the high-light-inducible proteins (HLIPs; also called SCPs) seem to protect the cells by heat dissipation of the absorbed energy, and mutants lacking these proteins are more sensitive to high light conditions (He et al., 2001; Havaux et al., 2003). The HLIPs that are synthesized in cyanobacterial cells grown under high-light conditions and other stress conditions (Dolganov et al., 1995; Funk and Vermaas, 1999; He et al., 2001) are chlorophyll binding single-helix polypeptides related to the LHC proteins and to the early-light-inducible proteins (Adamska, 1997, 2001).

Our recent results demonstrating the existence of the phycobilisome OCP–related NPQ mechanism in cyanobacteria grown in the presence of iron (Wilson et al., 2006) led us to consider the possibility that this mechanism could also play a role in the (blue) light-induced fluorescence quenching observed under iron starvation conditions. To date, only IsiA had been implicated in mediating this process. To test this idea, we studied light-induced fluorescence quenching in several iron-starved Synechocystis PCC 6803 mutants: a mutant without IsiA (ΔIsiA), a mutant without OCP (ΔOCP), and a mutant without phycobilisomes (PAL). Indeed, our results demonstrate that under iron starvation the blue-green light–induced NPQ mechanism is related to the OCP-mediated quenching of the phycobilisome emission and not to modifications of IsiA. Even though the presence of the IsiA protein protects the cells from stress, this protein is not involved in the light-induced NPQ mechanism.

**RESULTS**

**Pigment Changes during Iron Starvation in Wild-Type, ΔIsiA, and ΔOCP Mutant Cells**

Cyanobacteria grown under iron-deficient conditions exhibit decreased chlorophyll and PC content (Öquist, 1971, 1974a;
Guikema and Sherman, 1983; Sandmann, 1985). These changes are systemic; the levels of PSI, PSII (Spiller and Terry, 1980; Guikema and Sherman, 1983), and thylakoid (Sherman and Sherman, 1983) all decrease during iron starvation, with the decrease in PSI being the most marked (Guikema and Sherman, 1983; Sandmann, 1985). The presence of IsiA causes a blue shift in the room temperature chlorophyll a absorbance peak (680 to 673 nm), and the chlorophyll a fluorescence at 77K becomes dominated by a high emission at 685 nm (Öquist, 1974b; Burnap et al., 1993; Falk et al., 1995; Park et al., 1999). Odom et al. (1993) were the first to describe these changes in iron-starved Synechocystis PCC 6803.

Under experimental conditions of iron starvation and low-light intensities (see Methods), during the first 6 d, the content of chlorophyll per cell and the PC/chlorophyll ratio remained similar to those in unstarved wild-type, ΔOCP, and ΔIsiA Synechocystis PCC 6803 cells (Figure 1). Subsequently, the chlorophyll content of the wild-type and ΔOCP cells decreased; the maximum of the chlorophyll absorbance peak at 683 nm down-shifted until it reached 674 nm after 10 d of starvation (Figures 1A and 1B). In ΔIsiA cells, no shift of the chlorophyll-related peak was observed, and the chlorophyll content per cell decreased faster than in wild-type cells (Figures 1C and 1D). Even though the PC content per cell also decreased, an increase of the PC/chlorophyll ratio was observed in ΔIsiA cells (Figure 1C), while in wild-type and ΔOCP cells, the PC/chlorophyll ratio remained almost constant (Figures 1A and 1B). After 20 d of iron starvation, the ΔIsiA contained almost no chlorophyll and the cells were dead (Figure 1D). By contrast, wild-type and ΔOCP cells containing a low content of chlorophyll and a high content of carotenoids continued to survive and were still alive after 50 d of iron starvation (see Supplemental Figure 4 online).

Fluorescence Changes during Iron Starvation in Wild-Type and ΔIsiA Cells

Figure 2 shows the 77K fluorescence emission spectra of wild-type and ΔIsiA cells grown in complete medium or medium lacking iron for 7, 10, 12, and 14 d. Fluorescence excitation experiments using 430-nm light, which is preferentially absorbed by chlorophyll, and 600-nm light, which is preferentially absorbed by the phycobilisomes, were used to monitor the changes in the photosynthetic apparatus induced by iron starvation. When wild-type and ΔIsiA cells grown in iron-containing medium were excited at 430 nm, the 77K fluorescence spectra showed bands at 685 and 695 nm (related to the CP43 and the CP47 chlorophyll antenna of PSII, respectively; Van Dorssen et al., 1987; Siefermann-Harms, 1988), and a large PSII chlorophyll-related band at 725 nm (Figures 2A and 2B). In the early phases of iron starvation, wild-type and ΔIsiA cells showed an increase in the ratio of PSII fluorescence (685- and 695-nm peaks) to PSII fluorescence, suggesting an increased PSII/PSI ratio (Figures 2A and 2B). This is in accordance with the observation that the content of PSI, which contains three 4Fe-4S centers, decreases faster than that of PSII, which contains only three iron sulfur centers (Guikema and Sherman, 1983; Sandmann, 1985; Falk et al., 1995; Ivanov et al., 2000).

After prolonged (10 to 14 d) iron starvation, both strains showed a more rapid increase in the emission at 685 nm relative to that at 695 nm (Figures 2A and 2B). This was particularly marked in wild-type cells in which the 685-nm fluorescence emission reflects the presence of PSI-less IsiA aggregates (Yeremenko et al., 2004). PSI-IsiA supercomplexes have a very small 686-nm emission that makes a negligible contribution to this band (Andrizhiyevskaya et al., 2002; Yeremenko et al., 2004). The presence of IsiA and IsiA aggregates was confirmed by protein gel blot experiments (Figure 3). IsiA was present in 10 d iron-starved wild-type cells, but it was absent in wild-type cells grown in the presence of iron and in iron-starved ΔIsiA cells. The amount of CP47 (a measure of PSII) was increased in cells under iron-starved conditions relative to cells grown in the presence of iron (Figure 3). In iron-starved ΔIsiA cells, the amount of CP47 was even slightly higher than in wild-type cells. These results were in accordance with the increased PSII/PSI ratio deduced from Nonstarved and Starved Synechocystis Wild-Type and Mutant Cells.

Coomassie blue-stained gel electrophoresis and immunoblot detection of IsiA and CP47 in the membrane fractions isolated from 10 a iron-starved ΔIsiA (lane 1) and wild-type (lane 2) cells and nonstarved wild-type cells (lane 3). The IsiA antibody also reacts with IsiA aggregates (IsiA agg). Lane 4, molecular mass markers (in kilodaltons). Each sample contained 1 µg of chlorophyll.
from fluorescence spectra and variable fluorescence measurements (see below). Dühring et al. (2006), using Blue-Native PAGE gels, has also shown that in ΔIsiA iron-starved cells there is a higher concentration of PSII dimer and monomer than in wild-type iron-starved cells.

In iron-starved ΔIsiA cells, the increase of the 685-nm emission is evidently not associated with IsiA; instead, the source of this emission may be the phycobilisome terminal emitter, the Lcm, in disconnected phycobilisomes (see Supplemental Figure 1 online). In long-term iron-starved wild-type cells, a contribution of the emission from Lcm of uncoupled phycobilisomes may also be present.

In cyanobacteria, the fluorescence spectrum generated at 77K by 600-nm excitation contains emission bands related to PC (650 nm), APC (660 nm), PSII (685 and 695 nm), and PSI (725 nm) (Figures 2 C and 2D). The peak at 695 nm derives from the chlorophyll a emission of the PSII antenna CP47 and the reaction center. The emission at 685 nm is principally related to the phycobilisome terminal emitter, but fluorescence emission from the CP43 PSII antenna also contributes to this peak. When empty (without PSI) IsiA complexes are present, their fluorescence emission significantly contributes to the 685-nm peak. In the fluorescence spectra, the first manifestation of iron starvation in wild-type and ΔIsiA cells was an increase in the ratio of PSII fluorescence (685- and 695-nm peaks) to PSI fluorescence (Figures 2 C and 2D). Extended iron starvation resulted in a large increase of the 685-nm peak. This increase was faster and more pronounced in ΔIsiA cells than in wild-type cells (Figures 2 C and 2D). The origin of this increase was different in each strain. In ΔIsiA cells, this increase can only be related to the phycobilisome terminal emitter, reflecting an accumulation of functionally disconnected, high fluorescent phycobilisomes. In wild-type cells, the increase could be attributed to IsiA emission (Yeremenko et al., 2004) due to energy transfer from the phycobilisomes to IsiA complexes, as suggested by Joshua et al. (2005), and to a small population of uncoupled phycobilisomes in long-term iron-starved cells.

This suggestion was confirmed by comparison of 77K excitation fluorescence spectra of the emissions at 725 (PSI emission), 698 (PSII emission), and 683 nm (IsiA aggregates and phycobilisome emission) in iron-starved wild-type and iron-starved ΔIsiA cells. Isolated phycobilisomes also have a relatively high emission at 698 and 725 nm (see Supplemental Figure 2 online). In iron-starved wild-type cells, the spectra showed higher contributions from chlorophyll a (peaks at around 435 and 680 nm) and smaller contributions from components at 570, 620 (from PC), and 650 nm (from APC) than in iron-starved ΔIsiA cells (Figure 4). Moreover, the relative contribution of PC to the spectra increased with the increase of the 685-nm emission peak in iron-starved cells. These results indicated that in ΔIsiA cells, a large

Figure 4. The 77K Excitation Fluorescence Spectra of Iron-Starved Wild-Type and ΔIsiA Cells.
quantity of functionally disconnected phycobilisomes was present. The spectra also strongly suggest that in iron-starved wild-type cells, most of the phycobilisomes transferred the absorbed energy not only to the photosystems but also to IsiA complexes (see Supplemental Figure 2 online).

Phycobilisome-associated membrane fractions (MPs) from nonstarved and 11 or 15 d iron-starved wild-type and ΔIsiA cells were prepared to elucidate if the functionally disconnected phycobilisomes remained associated with the thylakoids. The cells were broken in a phosphate/citrate buffer. After discarding unbroken cells, the MPs were obtained by centrifugation. In all cases, the supernatant was nearly colorless, indicating that the bulk of the phycobilisomes remained attached to the thylakoids. This was confirmed by absorption spectra (see Supplemental Figure 3 online). The PC/chlorophyll ratio was higher in ΔIsiA MP compared with that of MP from wild-type cells. Fifteen days iron-starved ΔIsiA cells and MPs showed high and similar fluorescence at 685 nm. The magnitude of this fluorescence suggested that already in whole cells a large quantity of phycobilisomes (perhaps nearly all) were functionally disconnected. The rest of the MP preparations produced a larger emission at 685 nm than whole cells, suggesting a compromised connection between the phycobilisomes and the thylakoids in vitro (Figure 5). Nevertheless, as in whole cells, ΔIsiA MPs presented a larger 685-nm emission than wild-type MPs (Figures 5B and 5D). Thus, we conclude that in ΔIsiA iron-starved cells, all the phycobilisomes were attached to the thylakoids as in wild-type cells, but a larger number were functionally disconnected; they did not transfer energy to any chlorophyll complex.

Fluorescence Quenching in Iron-Starved Wild-Type and ΔIsiA Cells

The induction of the blue light–induced fluorescence quenching was monitored using a pulse-amplitude modulated fluorimeter (PAM). In cyanobacteria, the fluorescence detected by a PAM fluorometer is emitted from chlorophyll and phycobiliproteins (Campbell et al., 1998). In the PAM fluorometer, the measuring light has a maximum of excitation at 650 nm, and the fluorescence is detected at wavelengths beyond 700 nm. In cyanobacteria, which lack chlorophyll b, most of the measuring light is absorbed by the phycobilisomes. Thus, Fo, the minimal fluorescence level in dark-adapted cells, varies according to the cellular phycobiliprotein concentration (e.g., very low in mutants without phycobiliproteins) (Campbell et al., 1998; El Bissati and Kirilovsky, 2001). The Fo level also depends on the coupling of phycobilisomes: energetically coupled phycobilisomes show
low-yield fluorescence emission, while uncoupled phycobilisomes show high-yield fluorescence emission. Figures 6A to 6C compare the room temperature fluorescence traces measured with a PAM fluorometer after 0, 7, 10, 12, and 14 d of iron starvation from wild-type and ΔisiA cells. Dark-adapted cells were successively illuminated by dim and strong blue-green light. The cells under dim blue-green light, which preferentially excites PSI, showed a high level of fluorescence characteristic of State 1, which is induced by oxidation of the plastoquinone pool upon illumination of dark-adapted cells. Subsequently, exposure of cells to strong blue-green light induced the quenching of all levels of fluorescence (Fm', Fs, and Fo) in both wild-type and ΔisiA cells. The fluorescence quenching increased as iron starvation was prolonged. Figure 6D shows the increase of NPQ [(Fm' − Fm)/Fm'] values during iron starvation in wild-type and ΔisiA cells. This increase was faster and more marked in ΔisiA cells.

We also observed that after 6 to 7 d of iron starvation, differences in Fo values and Fv/Fo or Fv/Fm ratios appeared. First, in both strains at equimolar chlorophyll concentrations, Fo and Fv increased in parallel, and the ratios Fv/Fo and Fv/Fm remained high (even slightly higher than in nonstarved cells) (Figure 6, Table 1), suggesting that the increase of Fo and Fv was due to an increase in the PSII/PSI ratio, in agreement with the 77K fluorescence spectra and the observation that the per cell PSI content decreased faster than PSII content. With prolonged iron starvation, Fv began to decrease while Fo continued to increase, indicating a loss of active PSII (Figure 6, Table 1). In ΔisiA cells, this process was faster. After 14 d of Fe starvation, Fo was very small, while it was still relatively large in wild-type cells (Figure 6, Table 1). Wild-type cells were still alive after 48 d of iron starvation and had partially recovered the lost Fv, while ΔisiA cells died after 18 d of iron starvation (see Supplemental Figure 4 online).

In ΔisiA cells, the large increase of Fo can be explained by a rapid increase of a population of energetically uncoupled phycobilisomes detected in 77K fluorescence spectra. In wild-type cells, the increase of Fo could be related not only to an increase of fluorescence emitted by the uncoupled phycobilisome population but also to fluorescence emitted by IsiA complexes. In wild-type cells, the presence of IsiA protects the cell (previously shown in Park et al., 1999; Sandstro¨ m et al., 2001; Havaux et al., 2005) from the stress generated by iron starvation (Havaux et al.,

![Figure 6. Blue-Green Light–Induced Fluorescence Quenching in Iron-Starved Wild-Type and ΔisiA Cells.](image)

(A) to (C) The 0 d (green), 7 d (data not shown; similar to 0 d), 10 d (red), 12 d (blue), and 14 d (black) iron-starved ΔisiA (A) and wild-type (B) and (C) fluorescence traces in 7 and 14 d iron-starved wild-type cells are shown with a different scale than (A) and (B) to clarify the differences in fluorescence quenching. (D) Increase of NPQ [(Fm' − Fm)/Fm'] during iron starvation of wild-type (circles and solid line) and ΔisiA (squares and dotted line) cells. The graph is the average of four independent experiments. Error bars show the maximum and minimum NPQ values for each point.
Phycobilisome or Chlorophyll Emission Quenching?

As stated above, a decrease in the fluorescence levels observed in a PAM fluorometer could be a result of (1) a diminution of the phycobilisome emission, (2) a decrease in the chlorophyll antenna emission, or (3) a decrease in energy transfer from the phycobilisomes to PSII. We have already demonstrated that in *Synechocystis* PCC 6803 grown in iron-containing medium, the blue-green light–induced fluorescence decrease observed in the PAM fluorometer is due to the quenching of the phycobilisome fluorescence emission and a concomitant decrease in the energy transfer from the phycobilisomes to the photosystems (Wilson et al., 2006).

Room temperature fluorescence spectra were used to elucidate the origin of the fluorescence quenching in iron-starved cells. When cells iron-starved for 12 d were excited at 600 nm (light principally absorbed by phycobilisomes), the peak at 660 nm (phycobilisome-related) was more pronounced in iron-starved ΔIsiA cells than in iron-starved wild-type cells (Figures 8A and 8B). The large 660-nm fluorescence in ΔIsiA cells is most probably the result of disconnected phycobilisomes (see also Figure 2D). In iron-starved wild-type cells, an increase of the shoulder at 680 nm (chlorophyll related) compared with non-starved cells was observed (see Supplemental Figure 5 online). This was attributed to the presence of IsiA and to energy transfer from the phycobilisomes to IsiA. In iron-starved wild-type and ΔIsiA cells that were illuminated with strong blue-green light for 5 min (quenched cells), a very large decrease of the 660-nm band was observed (Figures 8A and 8B). Figure 8C shows that in wild-type cells iron-starved for an extended period (48 d), the 660-nm fluorescence emission increased and the fluorescence quenching was larger.

When iron-starved wild-type cells were excited at 430 nm (light principally absorbed by chlorophyll), a large band at 680 nm was observed, corresponding to the accumulation of IsiA complexes. This chlorophyll-related band was not decreased after illumination of iron-starved wild-type cells with strong blue light (Figures 8D, 12 d, and 8F, 48 d). In iron-starved ΔIsiA cells, even when the cells were excited at 430 nm, a relatively large emission at 680 nm (a phycobilisome related band) was observed (Figure 8E; see Supplemental Figure 5 online). A decrease of this emission was induced by strong blue-green light illumination. In conclusion, room temperature fluorescence spectra of unquenched and quenched iron-starved wild-type and ΔIsiA cells strongly suggested that under iron starvation conditions the blue-green-induced fluorescence quenching was attributable to a decrease of phycobilisome fluorescence emission and a concomitant decrease in the energy transfer from the phycobilisome to the photosystems and to IsiA (in the wild type).

To elucidate if there was a relationship between the larger NPQ in iron-starved cells relative to nonstarved cells and to the quantity of OCP present, protein gel blot analyses were undertaken. Total cellular proteins or MP proteins were separated by SDS-PAGE, and the OCP was detected by an anti-OCP antibody (Figure 9). The comparison was done on a per chlorophyll basis because all the fluorescence experiments were performed at the same chlorophyll concentration. The antibody reacts with a 35-kD polypeptide absent in the ΔOCP mutant. The immunoreaction was more pronounced in iron-starved cells and their MP fractions than in non-iron-starved cells and their MP fractions (per chlorophyll). Moreover, there was more OCP in ΔIsiA iron-starved cells and MP fractions (per chlorophyll) than in wild-type iron-starved cells and MP fractions (Figure 9).

### Table 1. Changes in Fluorescence Levels during Iron Starvation in Wild-Type and ΔIsiA Cells

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<thead>
<tr>
<th>Type</th>
<th>0 d Wild-Type/ΔIsiA</th>
<th>7 d Wild-Type/ΔIsiA</th>
<th>10 d Wild-Type/ΔIsiA</th>
<th>12 d Wild-Type/ΔIsiA</th>
<th>14 d Wild-Type/ΔIsiA</th>
<th>19 d Wild-Type/ΔIsiA</th>
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<tr>
<td>Fo (% ± 10%)</td>
<td>100/100</td>
<td>100/105</td>
<td>125/160</td>
<td>154/350</td>
<td>180/642</td>
<td>260/~</td>
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<tr>
<td>Fv (% ± 10%)</td>
<td>100/100</td>
<td>100/147</td>
<td>140/187</td>
<td>148/183</td>
<td>120/27</td>
<td>30/~</td>
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<tr>
<td>Fv/Fo (±0.05)</td>
<td>0.78/0.78</td>
<td>0.76/1.1</td>
<td>0.80/0.95</td>
<td>0.74/0.4</td>
<td>0.5/0.035</td>
<td>0.09/~</td>
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<tr>
<td>Fv/Fm (±0.05)</td>
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<td>0.43/0.50</td>
<td>0.47/0.47</td>
<td>0.43/0.3</td>
<td>0.33/0.03</td>
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Each result is the average of four independent iron starvation experiments.

2005; Latifi et al., 2005). Alone, in ΔIsiA cells, the blue light–induced NPQ mechanism seems to be insufficient protection from this specific stress.

When the iron-starved, quenched, wild-type, and ΔIsiA cells were exposed to dim blue-green light in the presence of chloramphenicol, an inhibitor of protein synthesis, they recovered their maximal level of Fm′ (Figure 7). These results confirmed that blue-green light–induced fluorescence quenching (in both iron-starved strains) was not related to photoinhibition/D1 damage. Similar effects were seen in comparable experiments in wild-type cells grown in the presence of iron (El Bissati et al., 2000; Wilson et al., 2006).

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<td>Fo (% ± 10%)</td>
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<td>30/~</td>
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<tr>
<td>Fv/Fo (±0.05)</td>
<td>0.78/0.78</td>
<td>0.76/1.1</td>
<td>0.80/0.95</td>
<td>0.74/0.4</td>
<td>0.5/0.035</td>
<td>0.09/~</td>
</tr>
<tr>
<td>Fv/Fm (±0.05)</td>
<td>0.44/0.44</td>
<td>0.43/0.50</td>
<td>0.47/0.47</td>
<td>0.43/0.3</td>
<td>0.33/0.03</td>
<td>0.085/~</td>
</tr>
</tbody>
</table>
In PAL cells grown in iron-containing medium, high intensities of blue-green light were unable to induce any fluorescence quenching (Wilson et al., 2006; Figure 10B). Figure 10B shows that this is also the case with PAL cells grown under iron starvation conditions. Since OCP is present in the PAL mutant (Figure 9), the lack of fluorescence quenching is due to the lack of phycobilisomes.

As with iron-starved wild-type cells, PAL cells showed changes in chlorophyll content and Fo and Fv values during iron starvation. During the first days of iron starvation, the chlorophyll content decreased to 60% of that of nonstarved cells and then it remained constant. During this time, Fv increased 1.5 times compared with nonstarved PAL cells, suggesting a larger number of active PSII centers on a chlorophyll basis (Figures 10B and 10C). The increase of Fo was higher (2.2 times), suggesting that part of the Fo increase was due to fluorescence emitted by IsiA complexes. As the duration of iron starvation increased, Fo remained almost constant and Fv slowly decreased to a slightly slower value than that in nonstarved cells (Figure 10C). The cells were still alive after 1 month of iron starvation.

The chlorophyll content and the PC/chlorophyll ratio of ΔOCP cells under iron starvation conditions were similar to that of wild-type cells (Figures 1A and 1B). Likewise, Fo and Fv values were similar in wild-type and ΔOCP cells grown in iron-containing medium. However, in general, the Fo level was higher in the wild-type than in ΔOCP iron-starved cells, while the Fv value was larger in ΔOCP than in wild-type cells (Figures 11A and 11B; see Supplemental Figure 4 online).

When the cells were excited at 600 nm, the emission peak at 685 nm was consistently smaller in ΔOCP relative to wild-type cells, suggesting a smaller quantity of uncoupled phycobilisomes in the ΔOCP iron-starved cells (Figure 11E). This was confirmed by comparison of 77K excitation fluorescence spectra of the emission at 685 nm in iron-starved wild-type and iron-starved ΔOCP cells. In iron-starved ΔOCP cells, the spectrum showed higher contributions from chlorophyll a (peaks at ~435 nm) and smaller contributions from components at 570, 620 (from PC), and 650 nm (from APC) than in iron-starved wild-type cells (Figure 11F). In iron-starved PAL cells, as expected, the major contribution to the excitation spectrum came from chlorophyll a (435 nm) (Figure 11F).

In general, fluorescence emission spectra at 77K using 430-nm excitation showed a larger 685-nm peak, suggesting a larger population of IsiA complexes in ΔOCP than in wild-type cells (Figure 12A). This was confirmed by protein gel blot experiments (Figure 12B). The iron-starved ΔOCP cells contained ~1.4 to 1.6 times more IsiA than iron-starved wild-type cells.

Measurements of fluorescence yield by a PAM fluorometer in iron-starved wild-type [A] and [C] and ΔIsiA [B] and [D] cells for 7 [A] and [B] and 14 d [C] and [D] at 3 μg chlorophyll/mL illuminated successively with low-intensity blue-green light (400 to 550 nm; 80 μmol photons m⁻² s⁻¹) and high-intensity blue-green light (740 μmol photons m⁻² s⁻¹) and then again with dim blue-green light. Chloramphenicol was present during all experiments. The figure shows a representative experiment.

**Figure 7.** Blue Light–Induced Quenching in Iron-Starved Wild-Type and ΔIsiA Cells Is Reversible without Protein Synthesis.
Figures 11A and 11B show the fluorescence traces in 14 d iron-starved wild-type and $\Delta OCP$ cells. In the absence of the OCP, blue-green light did not induce any fluorescence quenching (Figure 11B). Room temperature fluorescence spectra confirmed the absence of blue-green light–induced fluorescence quenching (Figures 11C and 11D). Figure 11D clearly demonstrates that blue light did not induce any quenching of chlorophyll fluorescence, which in the iron-starved $\Delta OCP$ cells is principally related to IsiA aggregates. In conclusion, under low light growth conditions, the presence of IsiA was sufficient to protect the $\Delta OCP$ cells, despite the absence of the blue light–induced NPQ mechanism.

DISCUSSION

When cyanobacteria grow under iron-limited conditions, the expression of IsiA, encoded by the isiA gene (Laudenbach and Straus, 1988), is synthesized in abundance. IsiA, which is a chlorophyll binding protein closely related to CP43, acts as an LHC of PSI under iron-deficient conditions (Bibby et al., 2001; Boekema et al., 2001). On the basis of results with a $\Delta$isiA of Synechococcus elongatus 7942 and a mutant of Synechococcus elongatus 7942 overproducing IsiA, a protective function of IsiA against photoinduced damage was suggested with IsiA acting as a dissipator of energy (Park et al., 1999; Sandström et al., 2001). Subsequent experiments using isolated IsiA complexes showed that these complexes are indeed efficient energy dissipators (Ihalainen et al., 2005).

It was proposed that this protection was associated with a strong blue (or white) light–induced NPQ mechanism: strong light could induce conformational changes of IsiA that transform IsiA complexes into energy dissipators (Cadoret et al., 2004) or modify the affinity of IsiA for phycobilisomes (Joshua et al., 2005). Our results clearly demonstrate that IsiA is not involved in a light-induced NPQ mechanism. In iron-starved cells (just as in iron-containing cells), the blue light–induced fluorescence quenching is associated with the phycobilisomes and with the OCP and not with IsiA. In the $\Delta$isiA mutant, a large reversible fluorescence quenching was always induced by blue light. By contrast, in mutants lacking phycobilisomes (PAL mutant) or OCP ($\Delta OCP$ mutant), blue light was unable to induce fluorescence quenching, even after very long periods of iron starvation. In addition, in iron-starved wild-type and $\Delta$isiA cells, fluorescence emission spectra showed that no chlorophyll-related fluorescence quenching was induced, while a large decrease of the fluorescence emitted by phycobilisomes and of the energy transfer from phycobilisomes to chlorophyll complexes was detected.
Blue light–induced quenching was observed in wild-type and ΔIsiA iron-containing cells (El Bissati et al., 2000; Wilson et al., 2006) but was more marked in iron-starved cells (Cadoret et al., 2004; this article). Moreover, during iron starvation, the increase in fluorescence quenching was faster in ΔIsiA cells than in wild-type cells. In iron-starved ΔIsiA cells, a rapid increase of the 685-nm peak in 77K fluorescence emission spectra, of the 660-nm peak in room temperature fluorescence spectra, and of the Fo level indicated a very fast increase of a population of functionally disconnected highly fluorescent phycobilisomes. In wild-type cells, the number of disconnected phycobilisomes also increased, but more slowly than in ΔIsiA cells. Thus, our results clearly reveal a relationship between the increase of fluorescence quenching and the number of functionally disconnected phycobilisomes. Cells containing a higher number of disconnected phycobilisomes showed a higher level of thermal energy dissipation (NPQ), thereby protecting themselves by diminishing the energy arriving at the photosystems and the thylakoids. The increased NPQ is associated with a higher concentration of the OCP: a higher concentration of the OCP was observed in iron-starved cells compared with nonstarved cells. The abundance of OCP is even greater in iron-starved ΔIsiA cells. The transcription of the slr1963 gene is known to be increased under other stress conditions: high white light illumination (Hihara et al., 2001), UV-B light (I. Vass, personal communication), and saline stress (Fulda et al., 2006). It is possible that other stresses also upregulate the expression of this gene. It may be that the OCP-related NPQ mechanism plays a significant protective role under a range of stress conditions.

In ΔOCP cells under prolonged iron starvation, blue light was unable to induce any fluorescence quenching even in the presence of functionally disconnected phycobilisomes and empty IsiA complexes. Thus, the OCP is essential for the NPQ occurring under iron starvation conditions. Interestingly, in iron-starved ΔOCP cells, the increase in the amount of uncoupled phycobilisomes and the decrease of Fv were slower than in wild-type cells. This appears to be the result of a higher concentration of IsiA. Increased IsiA concentration could be explained by the fact that ΔOCP cells, being more sensitive to light, are permanently under a greater oxidative stress than wild-type cells. It has already been demonstrated that oxidative stress induces isiA transcript accumulation (Jeanjean et al., 2003; Yousef et al., 2003). Singh et al. (2005) have shown that ΔIsiA cells were slightly more resistant to the presence of H2O2 than wild-type cells. They proposed that this higher resistance is related to the greater transcription induction of a gene cluster, including a gene encoding a peroxiredoxin that is involved in the detoxification of starved wild-type and ΔIsiA whole cells. The results represent the average of four independent experiments. Error bars show the maximum and minimum density of band values for each point.

Figure 9. OCP Detection in Whole Cells and MP Fractions from Non-starved and Starved Wild Type and Mutants.
(A) Coomassie blue–stained gel electrophoresis and immunoblot detection (bottom panel) of OCP in 12 d iron-starved ΔIsiA (lane 1) and wild-type (lane 2) cells and nonstarved ΔIsiA (lane 3), wild-type (lane 4), PAL (lane 6), and ΔOCP (lane 7) cells. Lane 5 shows molecular mass markers. Each lane contained 1.5 μg of chlorophyll.
(B) Comparative densitometry of OCP bands in nonstarved and iron-starved Whole Cells and MP Fractions from Non-starved and Starved Wild Type and Mutants.
(C) Coomassie blue–stained gel electrophoresis and immunoblot detection (bottom panel) of OCP in MP fractions isolated from 12 d iron-starved ΔIsiA (lane 1) and wild-type (lane 2) cells and nonstarved wild-type cells (lane 4). Lane 3 shows molecular mass markers. Each lane contained 1 μg of chlorophyll.
peroxide. Thus, under stress conditions, ΔOCP cells can synthesize not only IsiA but also other proteins (e.g., HLIPs, catalases, and peroxidases) to try to compensate for the lack of the phycobilisome-related NPQ mechanism. This could explain why ΔOCP cells were slightly more resistant to iron starvation than wild-type cells.

By comparing the effect of iron starvation in ΔIsiA cells to that in ΔOCP cells, we conclude that even though the phycobilisome-related NPQ mechanism is an important photoprotective process under high light conditions, under iron starvation (under low light conditions), the IsiA-related mechanism provides more effective protection of the cells. Under low light growth conditions, the phycobilisome-related NPQ mechanism is not induced. We have already shown that this mechanism is only induced by high intensities of blue or white light (Wilson et al., 2006). Thus, even if the capacity of induction of the phycobilisome-related NPQ mechanism is increased during iron starvation, this will not protect the cells from the iron starvation stress. However, in the iron-starved cells that are more sensitive to photoinhibition, the synthesis of OCP and the capacity of quenching are increased, providing greater photoprotective capacity under high light.

The blue light-induced fluorescence quenching is clearly not associated with the binding of free phycobilisomes to IsiA or to conformational changes in the IsiA protein that might cause the protein to become a more efficient thermal energy dissipator.

Since the absence of IsiA renders Synechocystis cells more sensitive to the stress generated by iron starvation and high light (Park et al., 1999; Havaux et al., 2005; Latifi et al., 2005; this article) but does not inhibit the NPQ process, IsiA protects the cells via other mechanism(s). Combining our observations with results presented in the literature, we posit that during the first days of iron starvation, IsiA protects the cells by acting as the LHC of PSI, increasing the cross section of the PSI antenna (Andrizhiyevskaya et al., 2002; Melkozernov et al., 2003). In this way, a higher activity of PSI is maintained and a balance between PSII and PSI activities, decreasing the excitation pressure on PSII and, hence, the oxidative stress. During prolonged iron starvation, empty IsiA complexes may receive the energy coming from phycobilisomes and convert it into heat, thereby decreasing the energy arriving at the PSII reactions centers and at the thylakoids. Isolated IsiA aggregates, which accumulate upon chronic iron starvation, show very short fluorescence lifetimes, indicating that they are good energy dissipators (Ihalainen et al., 2005).

We observed that the increase of the population of highly fluorescent phycobilisomes was inversely proportional to the concentration of empty IsiA complexes (very high in ΔIsiA cells and...
Figure 11. No Blue Light–Induced NPQ in Iron-Stressed ΔOCP Cells.

(A) and (B) Fluorescence changes in iron-starved ΔOCP cells. Dark-adapted 14 d iron-starved wild-type (A) and ΔOCP (B) cells were illuminated successively with low-intensity blue-green light (400 to 550 nm; 80 μmol photons m⁻² s⁻¹) and high-intensity blue-green light (740 μmol photons m⁻² s⁻¹). Fluorescence yield changes were detected in a PAM fluorometer. The cells were at 3 μg chlorophyll/mL.

(C) and (D) Room temperature fluorescence spectra of ΔOCP cells adapted to low light intensities of blue-green light (solid line) and after 5 min of high intensities of blue-green light illumination (dotted line). Excitation was done at 600 nm (C) and at 430 nm (D).

(E) The 77K fluorescence emission spectra of iron-containing (solid line), 14 d iron-starved (dotted line) wild-type cells, and 14 d iron-starved ΔOCP cells (dashed line). Excitation was done at 600 nm.

(F) The 77K fluorescence excitation spectra of 14 d iron-starved wild-type (solid line) and ΔOCP (dotted line) and PAL cells (dashed line). Emission was monitored at 685 nm. The cells were at 3 μg chlorophyll/mL.
lower in wild-type cells), suggesting that, indeed, the phycobilisomes transferred the absorbed energy to the IsiA complexes. This was supported by the fluorescence emission and excitation spectra of wild-type cells. The peak at 685 nm observed in fluorescence emission spectra (77K) with excitation at 600 nm was largely related to IsiA emission, indicating energy transfer from phycobilisomes to IsiA. Park et al. (1999) and Sandström et al. (2001) have already reported that the PSII antenna size was inversely proportional to the concentration of IsiA: the more IsiA present, the smaller the antenna size. Thus, IsiA complexes could protect PSII by competing for the energy coming from the phycobilisomes, diminishing the effective antenna size.

We suggest that the energy absorbed by the phycobilisomes that is not used for photochemistry could also be responsible for oxidative damage (e.g., by peroxidation of lipids). For example, Havaux et al. (2005) have already demonstrated that in ΔIsiA mutants, there is more lipid peroxidation and higher concentrations of reactive oxygen species are produced than in wild-type cells. This can be caused by a higher production of oxygen radicals in the chlorophyll antenna but also by production of carbon-centered radicals in other proteins of the membrane that by reacting with oxygen will make peroxyl radicals. Our results demonstrate that a mutant lacking phycobilisomes (PAL) seemed to be less affected by iron starvation conditions. In PAL cells, IsiA was accumulated and the PSII/PSI ratio increased, indicating a faster degradation of PSI complexes relative to PSII. However, a high concentration of active PSI complexes remained after a long period of iron starvation. These results support the idea that the presence of functionally disconnected phycobilisomes is deleterious to the cell.

**Conclusions**

The recently described light-induced photoprotective phycobilisome OCP–mediated NPQ mechanism also occurs under iron starvation conditions, where it is solely responsible for the light-induced fluorescence quenching observed in iron-starved cyanobacteria. In our working model for this process, blue-green light absorbed by the carotenoid of the OCP induces changes in the carotenoid and/or the protein that facilitates the interaction between the OCP and the phycobilisome core and renders the OCP capable of absorbing the energy arriving from the phycobilisomes and dissipates it as heat. Under iron starvation conditions, conditions in which the phycobilisomes become disconnected from the photosystems, energy dissipation in phycobilisomes increases (increased NPQ) to better protect the cells by diminishing the energy arriving to the thylakoids.

The empty IsiA complexes that accumulate during iron starvation could be responsible for a permanent form of thermal energy dissipation. However, this IsiA-based mechanism is not modulated by light. Ihalainen et al. (2005) have shown that the quenched state of the IsiA complexes was independent of the quality or intensity of light, at least in vitro. Under our experimental conditions, cells lacking IsiA were more sensitive to iron starvation than cells unable to activate blue light–induced NPQ. Thus, under iron starvation and low light conditions, IsiA complexes provide better protection than the phycobilisome OCP–related NPQ mechanism. The absence of IsiA and HLIPs, like the
absence of the OCP, renders *Synechocystis* PCC 6803 cells more sensitive to high light intensities. In wild-type cells, IsiA and HLIPs are present only under prolonged high light conditions, whereas the OCP is always present, suggesting that the IsiA- and HLIP-related mechanisms appear when the OCP-related NPQ mechanism is insufficient to protect the cell. A comparative study of the role and effectiveness in photoprotection of these proteins under high light conditions will increase our understanding of the different NPQ mechanisms existing in cyanobacteria.

**METHODS**

**Culture Conditions**

Wild-type and mutant *Synechocystis* PCC 6803 cells were grown photoautotrophically in a modified BG11 medium described by Herdman et al. (1973) containing twice the concentration of sodium nitrate. Cells were shaken in a rotary shaker (120 rpm) at 30°C and illuminated by fluorescent white lamps giving a total intensity of ~30 to 40 μmol photons m⁻² s⁻¹ under a CO₂-enriched atmosphere. The ΔOCP, ΔIsiA, and PAL mutants were grown in the presence of 25 μg/mL spectinomycin and 10 μg/mL streptomycin. The cells were maintained in the logarithmic phase of growth and were collected at OD₈₀₀ = 0.6 to 0.8. The construction of the ΔOCP and ΔIsiA mutants in which the *slr1963* gene and the *isiA* gene are interrupted by a spectinomycin/streptomycin resistance cassette was described by Wilson et al. (2006). Construction of the PAL mutant, lacking PC, APC, and the phycobilisome linker protein Lcm, was described by Ajlani and Vernotte (1998).

**Iron Starvation**

Wild-type and mutant *Synechocystis* PCC 6803 cells were collected at the logarithmic phase of growth, precipitated, resuspended (OD₈₀₀ = 0.6) in modified BG11 medium (Herdman et al., 1973) lacking Fe, and grown under low light (30 to 40 μmol photons m⁻² s⁻¹). During the first week, the cells were diluted each day to maintain the same cell concentration (OD₈₀₀ ranging from 0.6 in the morning to 1.2 to 0.9 the next morning). When the chlorophyll concentration per cell decreased, the cells were diluted once every 2 d, then once every 3 d, and finally undiluted. The PAL mutant was diluted only every 3 d consistently. When the cell concentration was maintained at less than OD₈₀₀ = 0.5, the phenotype of protractedly iron-starved cells was reached faster (in 6 to 7 d) (data not shown). This facilitated study of the different stages of iron starvation.

**Fluorescence Measurements**

The yield of chlorophyll fluorescence was monitored in a modulated fluorometer (PAM; Walz) adapted to a Hansatech oxygen electrode as previously described (El Bissati et al., 2000). All NPQ induction and recovery experiments were performed in a stirred cuvette of 1 cm diameter (32°C) at a chlorophyll concentration of 3 μg chlorophyll/mL (with the exception of the PAL mutant, 2 μg chlorophyll/mL) and in the presence of chloramphenicol (30 μg/mL) to inhibit protein synthesis. Recovery was realized by illuminating dark-adapted cells with a low intensity of red-modulated light (pulses of 1 μs, 1.6 kHz, and 0.024 μmol photons m⁻² s⁻¹). For PAL measurements, the measuring light was 2.5 times more intense. Saturating pulses (2000 μmol photons m⁻² s⁻¹, 1 s) were applied to measure Fm and Fm’ levels. Application of such pulses that transiently close all the PSIi centers serves to distinguish between photochemical quenching (qp) and NPQ.

Fluorescence emission and excitation spectra at room temperature and at 77K were done in a CARY Eclipse fluorescence spectrophotometer fluorometer (Varian). All samples were at a concentration of 2 to 3 μg chlorophyll/mL. For 77K spectra, samples in nuclear magnetic resonance tubes (5-mm ratio) were quickly frozen by immersion in a mixture of ice, CO₂, and ethanol and then in liquid nitrogen.

**MP, MP-Free, and Phycobilisome Preparations**

Cells (at a 1 mg chlorophyll/mL concentration) were resuspended in a 0.7 M K-phosphate/0.3 M Na-citrate, pH 6.8, buffer to obtain MP or in a 20 mM MES, pH 6.8, buffer to obtain MP-free (M) and broken in a mini-bead-beater in the presence of glass beads. The M and MP fractions were collected by centrifugation and frozen at ~80°C until used for gel electrophoresis. For fluorescence spectra, the MP fraction was immediately used without freezing. The PC/chlorophyll absorption ratio was similar in MP fractions and in whole cells.

Entire phycobilisomes were isolated as described by Ajlani et al. (1995). Cells were broken in a buffer containing 0.75 M K-phosphate, pH 7.5, and treated with Triton X-100 for 1 h. The nonsolubilized material was separated by centrifugation, and the supernatant was applied to a sucrose gradient and centrifuged. The blue band (in the interface between 1.5 M sucrose and 0.75 M sucrose) contained intact phycobilisomes used in fluorescence spectra.

**Gel Electrophoresis and Protein Gel Blot Analysis**

Total cell protein and MP and M fractions were analyzed by SDS-PAGE on a 12% polyacrylamide/2 M urea gel (Figures 3A and 12B) or on a 12% Tris/MES system (Figures 3 and 9C). For 77K spectra, samples in nuclear magnetic resonance tubes (5-mm ratio) were quickly frozen by immersion in a mixture of ice, CO₂, and ethanol and then in liquid nitrogen.

**Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers NP_441508 (slr1963) and NP_441288 (isiA; sili24).**

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure 1.** The 77K Emission and Excitation Fluorescence Spectra of Starved ΔIsiA Cells and Isolated Phycobilisomes.

**Supplemental Figure 2.** The 77K Emission and Excitation Fluorescence Spectra of Nonstarved Wild-Type Cells, the MP Fraction, and Isolated Phycobilisomes.
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Supplemental Figure 3. Absorption Spectra of Wild-Type and ΔIsiA Cells and MP Fractions Corresponding to the Samples Used in Figure 5.

Supplemental Figure 4. Blue-Green Light–Induced Fluorescence Quenching in Long-Term, Iron-Starved, Wild-Type, ΔOCP, and ΔIsiA Cells.

Supplemental Figure 5. Comparison of Room Temperature Fluorescence Spectra of Nonstarved and Iron-Starved Wild-Type and ΔIsiA Cells.

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