Mechanism of the Down Regulation of Photosynthesis by Blue Light in the Cyanobacterium *Synechocystis* sp. PCC 6803†

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ABSTRACT: Exposure to blue light has previously been shown to induce the reversible quenching of fluorescence in cyanobacteria, indicative of a photoprotective mechanism responsible for the down regulation of photosynthesis. We have investigated the molecular mechanism behind fluorescence quenching by characterizing changes in excitation energy transfer through the phycobilin pigments of the phycobilisome to chlorophyll with steady-state and time-resolved fluorescence excitation and emission spectroscopy. Quenching was investigated in both a photosystem II-less mutant, and DCMU-poisoned wild-type *Synechocystis* sp. PCC 6803. The action spectra for blue-light-induced quenching was identical in both cell types and was dominated by a band in the blue region, peaking at 480 nm. Fluorescence quenching and its dark recovery was inhibited by the protein cross-linking agent glutaraldehyde, which could maintain cells in either the quenched or the unquenched state. We found that high phosphate concentrations that inhibit phycobilisome mobility and the regulation of energy transfer by the light-state transition did not affect blue-light-induced fluorescence quenching. Both room temperature and 77 K fluorescence emission spectra revealed that fluorescence quenching was associated with phycobilin emission. Quenching was characterized by a decrease in the emission of allophycocyanin and long wavelength phycobilisome terminal emitters relative to that of phycocyanin. A global analysis of the room-temperature fluorescence decay kinetics revealed that phycocyanin and photosystem I decay components were unaffected by quenching, whereas the decay components originating from allophycocyanin and phycobilisome terminal emitters were altered. Our data support a regulatory mechanism involving a protein conformational change and/or change in protein−protein interaction which quenches excitation energy at the core of the phycobilisome.

The efficient harvesting of solar energy is the critical first step in photosynthesis. However, excess light can be harmful when the amount of absorbed light exceeds the capacity of photosynthetic reaction centers to utilize it. Photosynthetic organisms have evolved a number of photoregulatory processes (*1*). These include state transitions (qT[1]), high energy quenching (qE), and photoinhibitory quenching (qI). State transitions are a rapid rearrangement of the photosynthetic light-harvesting system in response to changing light conditions that serve to balance excitation between photosystem II (PSII) and photosystem I (PSI) to optimize photosynthetic electron transport, whereas qE and qI offer protection from photodamage when light absorption exceeds metabolic demand (*2*, *3*). Although details differ, all oxygenic photosynthetic organisms appear to have at least one type of mechanism that serves to regulate the amount of excitation energy reaching PSII and would fit broadly into one of these categories of nonphotochemical quenching.

In higher plants, the dissipation of excess light energy as heat (qE) is associated with the membrane-bound light-harvesting chlorophyll antenna complexes (LHCII) and is triggered by the formation of the carotenoid zeaxanthin (*4*−*6*) and the protonation of the PsbS protein (*7*). Cyanobacteria, which lack LHCII and PsbS, do not have a similar mechanism for qE. Cyanobacteria do have phycobilisomes to capture light, which are large extrinsic pigment−protein complexes consisting of rods and cores that are associated with the stromal surface of the thylakoid membranes (*8*, *9*). The excitation energy absorbed by the phycobilisome is transferred from a relatively shorter wavelength-absorbing rod pigment (phycocyanin) to a longer wavelength-absorbing core pigment (allophycocyanin) and then to the reaction centers via even longer wavelength terminal allophycocyanin emitters (*10*).

Although the mechanism of the state transition is well understood in higher plants and green algae, it is still somewhat enigmatic in cyanobacteria (*11*, *12*). Cyanobacteria clearly change the distribution of phycobilisome absorbed

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[1] Abbreviations: qT, state transitions; qE, high energy quenching; qI, photoinhibitory quenching; qN, nonphotochemical quenching; OCP, orange carotenoid protein; DCMU, 3-(3′,4′-dichlorphenyl)-1,1-dimethylurea; PSI, photosystem I; PSII, photosystem II.

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light between PSII and PSI during state transitions (*13*). Red algae also decrease the probability of energy transfer from phycobilisomes to PSII after selective illumination of PSII in what was originally thought to be a qT type mechanism (*14*, *15*) but was subsequently shown to occur via a qE type mechanism (*16*).

To add complexity to an increasingly complex field, an additional form of fluorescence quenching in cyanobacteria, induced by blue light, was described by El Bissati et al. (*17*). Strong blue light led to a slow, reversible fluorescence quenching, independent of state transitions, whose recovery did not require protein synthesis. Blue-light quenching in cyanobacteria was further characterized by Rakhimberdieva et al. (*18*), who determined an action spectrum for quenching in a PSII-less mutant of *Synechocystis* and proposed a carotenoid-based mechanism.

Carotenoids in cyanobacteria are associated with both water soluble proteins as well as integral membrane-bound pigment−protein complexes. The 35 kD water soluble orange carotenoid binding protein (OCP) has been well characterized (*19*, *20*). A mutant lacking the soluble orange carotenoid binding protein (OCP) was recently shown to be incapable of blue-light-induced fluorescence quenching (*21*). In the same work, the OCP-less mutant was also shown to be more sensitive to photoinhibition. Wilson et al. (2006) proposed a working model for blue-ight-induced fluorescence quenching in which light activated OCP induces energy dissipation through interaction with the phycobilisome core.

We have investigated the molecular mechanism of blue-light induced quenching in *Synechocystis* sp. PCC 6803 wild-type and a PSII-less mutant using a combination of steady-state and time-resolved fluorescence spectroscopy. Our results describe the changes in excitation energy transfer within the phycobilisome that accompany blue-light-induced fluorescence quenching and strongly support the hypothesis that the site of quenching is the core of the phycobilisome. Our work further indicates that blue-light quenching is unaffected by treatments (high phosphate buffers) previously shown to inhibit phycobilisome mobility but is dependent on changes in either protein conformation and/or protein−protein interactions that are inhibited by protein cross-linking with glutaraldehyde.

MATERIALS AND METHODS

A PSII impaired mutant was isolated during an attempt to generate an insertional inactivation mutation of the slr1515 gene in *Synechocystis* sp. PCC 6803. The slr1515 gene is a homologue of the ictB gene in *Synechocystis* sp. PCC 7942 which had been proposed to encode a HCO_3^- transporter (*22*). Although the resulting mutant, M8, showed no inhibition of HCO_3^- transport ability, it had lost PSII activity. Subsequent analysis showed an additional point mutation in the gene coding for the core antenna Chl *a* binding polypeptide CP47.

For polypeptide isolation, intact cells were broken with a French press and the thylakoid membranes resuspended in 50 mM Tricine buffer at pH 7.8 with 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 10 mM NaF, 1 mM benzamidine, and 1mM caproic acid. Protein concentration was determined with a Bio-Rad DC protein assay. The samples were centrifuged 15 000*g* for 20 min at 4 °C, and the enriched membrane fractions were solubilized in 60 mM Tris (pH 8.0), 1% glycerol (v/v), 4% SDS (w/v) (ratio of SDS/proteins was 4:1) and frozen at −80 °C until use for SDS−PAGE and immunoblotting. The proteins were separated in 15% polyacrilamide resolving gel containing 6 M urea, 0.66 M Tris at pH 8.8, and stacking gel containing 0.125 M Tris (pH 6.8) using a discontinuous Laemmli buffer system (*23*). Electrophoresis was performed at room temperature in a Mini Bio-Rad at 25 mA. The polypeptides separated by SDS−PAGE were stained by Coomasie Blue or transferred to nitrocellulose membranes and blotted with PsbA polyclonal antibody (anti-D1 protein obtained from Agri-Sera, Sweden; dilution 1:3000), PsaA anti-PSI (dilution 1:2000), and phycocyanin antibody (dilution 1:500).

Fluorescence emission and excitation spectra at 77 K and pulse amplitude modulated (PAM) room temperature fluorescence kinetics were determined as described previously (*12*). For some experiments the PAM spectrometer was modified by replacing the standard 655 nm modulated LED with one that had a peak emission at 620 nm. In these experiments the standard long pass (>710 nm) blocking filter on the PAM was replaced by a shorter wavelength long pass (>660 nm) blocking filter.

Action spectra were determined by measuring the fluorescence change, with the PAM fluorometer, induced by the illumination of intact cyanobacteria with a fixed light intensity (50 μEm^{-2} s^{-1} in the M8 mutant and 100 μEm^{-2} s^{-1} in the DCMU poisoned wild type) of 10 nm bandwidth monochromatic light from a 75 W Xe arc lamp dispersed by a $^1/_4$ m monochromator (ScienceTech, Missisauga, Ontario).

Room-temperature picosecond time-resolved fluorescence emission decay kinetics were determined and analyzed globally with a model of parallel decaying compartments as described previously (*24*).

The 77 K fluorescence emission spectra, excitation spectra, and picosecond time-resolved decay kinetics were measured from 100 μL samples in capillary tubes that had been quickly frozen by immersion in liquid N_2. The 100 μL dark-adapted or blue-light quenched samples were taken from a larger room temperature stirred sample that was continuously monitored with the PAM fluorimeter.

RESULTS

The M8 mutant of *Synechocystis* sp. PCC 6803 was incapable of autotrophic growth and showed no oxygen evolution activity in the presence of either bicarbonate or benzoquinone as the electron acceptor. There was also no evidence for full electron transport chain activity from hydroxylamine to methyl viologen in the M8 mutant (data not shown). Variable fluorescence was highly modified in the M8 mutant which showed no immediate fluorescence rise (F_M) in response to single or multiturnover saturating flashes (data not shown). Protein gels for thylakoid membranes isolated from M8 showed no evidence of PSII proteins, and western blots showed no evidence for the D1 protein of PSII in the M8 mutant, although it did contain the phycobilin protein phycocyanin c and the PSI polypeptide PsaA (Figure 1).

The PSII-less M8 mutant exhibited a light-induced quenching in room-temperature steady-state fluorescence intensity

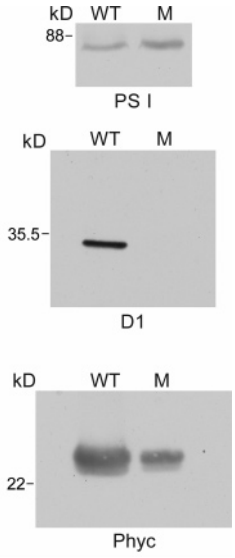

FIGURE 1: Western blots of polypeptides transferred to nitrocellulose after SDS gel electrophoresis; see Materials and Methods for details. Upper panel, blotted with PsaA antibody (anti-PSI); center panel, blotted with PsbA antibody (anti-PSII, D1 protein); lower panel, blotted with CpcC antibody (anti phycocyanin).

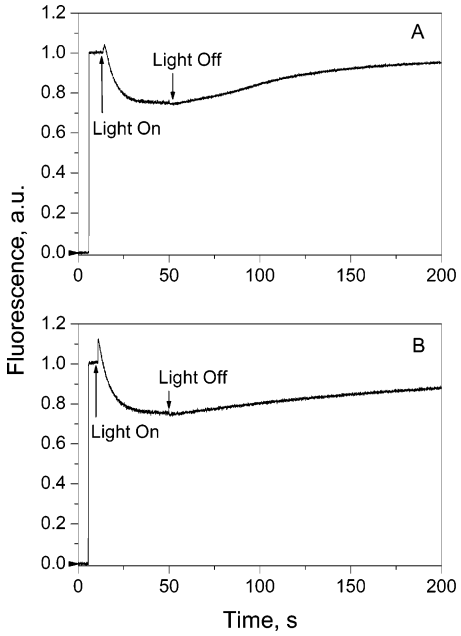

FIGURE 2: Pulse amplidude modulated fluorescence traces of M8 mutant cells and wild-type cells. (A) M8 mutant cells were dark-adapted for 5 min and then exposed to 100 μEm^{-2} s^{-1} of white light. (B) Wild-type cells in the presence of 20 μM DCMU were dark-adapted for 5 min and then exposed to 200 μEm^{-2} s^{-1} of white light. The measuring light intensity was less than 2 μEm^{-2} s^{-1} and did not induce any changes in fluorescence intensity on its own. The measuring light was generated by a 620 nm LED, and fluorescence was detected through a 660 nm long pass glass filter.

as had been observed previously in another PSII-less mutant (*18*). The quenching was reversible in the dark (Figure 2A). The light-induced changes in fluorescence observed in the mutant were unaffected by the addition of DCMU (data not shown).

Light-induced, DCMU-insensitive, reversible fluorescence quenching with very similar kinetics was also observed in wild-type *Synechocystis* sp. PCC 6803 cells (Figure 2B). The light intensities required to observe quenching in the wild-type were higher (~100 μEm^{-2} s^{-1}) than those required to induce quenching in the M8 mutant (~50 μEm^{-2} s^{-1}), although lower than the intensities previously reported in (*17*).

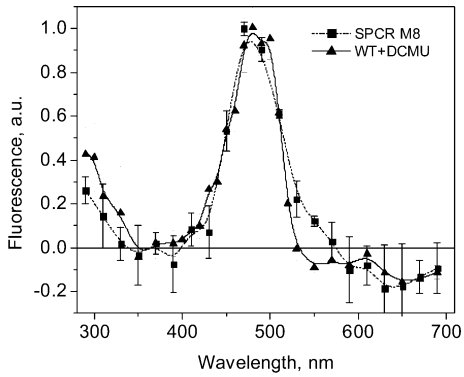

FIGURE 3: Action spectra for the blue-ight-induced fluorescence quenching in both the M8 mutant (■) and DCMU-treated wild type (▲). The data points are the average of three independent repeats, and the error bars show the standard deviation. See Materials and Methods for details.

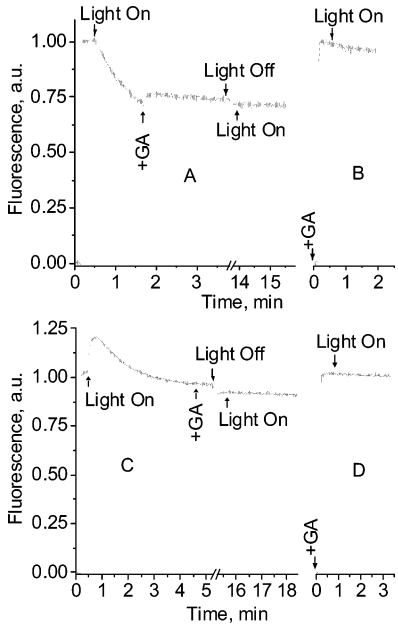

FIGURE 4: Effects of glutaraldehyde on blue-light-induced fluorescence quenching in (A, B) the M8 mutant and in (C, D) the wild type. Traces A and C show blue-light quenching, inhibition of quenching upon the addition of glutaraldehyde, and the cells treated with glutaraldehyde in the light. Traces B and D show the cells treated with glutaraldehyde in the dark. Glutaraldehyde was added to a final concentration of 0.5%. Room-temperature fluorescence was measured with a PAM fluorimeter.

Action spectra for the quenching were determined in both the M8 mutant and in the wild type in the presence of DCMU and are compared in Figure 3. For both cell types, the action spectrum peaks at approximately 480 nm and drops significantly beyond 520 nm. Quenching is also induced in both cell types by exposure to UV below 300 nm. These action spectra are very similar to that observed previously in a PSII-less mutant of *Synechocystis* sp. (*18*). As suggested by those authors, the action spectrum is reminiscent of a carotenoid absorption spectrum.

Figure 4 shows the effect of the protein cross-linking agent, glutaraldehyde, on fluorescence quenching in the M8 mutant

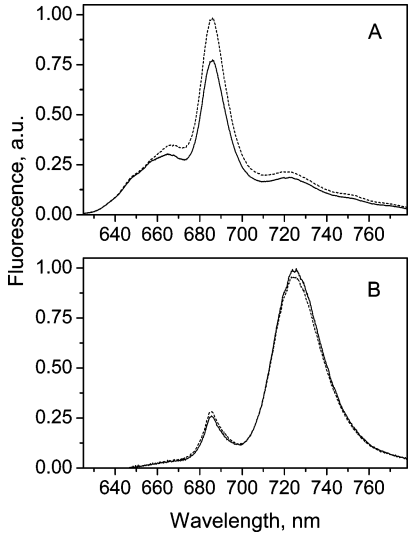

FIGURE 5: (A) 77 K fluorescence emission spectra for excitation at 590 nm and (B) 435 nm for M8 cells frozen in the blue-light quenched (___) and dark-adapted (----) states. The spectra have not been normalized and reflect real changes in fluorescence yield.

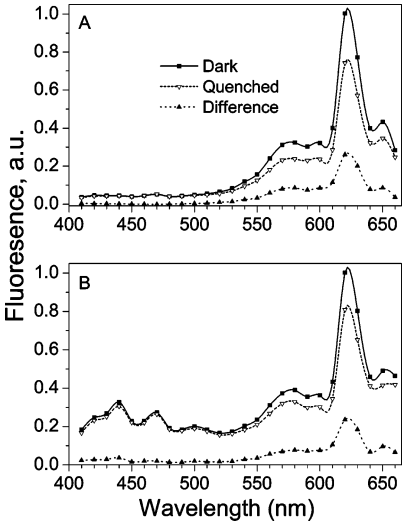

FIGURE 6: (A) 77K fluorescence excitation spectra for emission at 685 nm and (B) emission at 720 nm from M8 cells frozen in the blue-light quenched and dark-adapted states.

and in the wild type. The addition of glutaraldehyde during blue-light illumination inhibits further quenching (Figure 4A and C), if glutaraldehyde is added in the dark, the fluorescence remains high and unquenched upon subsequent exposure to light (Figure 4B and D). If glutaraldehyde is added while the fluorescence is quenched, the fluorescence level remains low and does not recover in the dark or change further in the light. The manifestation of the quenching and its recovery thus appear to involve changes in the association or conformation of proteins accessible to glutaraldehyde.

High osmotic conditions have been previously shown to inhibit state transition in cyanobacteria (*25*). We also found that suspension of cells in 0.5 M phosphate buffer inhibited state transition in wild-type cells (data not shown). However, 0.5 M phosphate had no effect on the blue-light-induced quenching in DCMU treated wild-type cells or in the M8 mutant (data not shown). The quenching was, however, irreversible in the presence of 0.5 M phosphate. This is consistent with the previous observation (*26*) that blue-ight-induced quenching in Fe starved cyanobacteria was not inhibited but irreversible in 1 M phosphate.

Fluorescence emission spectra were determined to explore the origin of fluorescence quenching. The 77 K fluorescence emission spectra of the M8 mutant cells frozen in the unquenched and the quenched states are shown in Figure 5. These spectra were not normalized, and they show real changes in the yield of emission between the quenched and unquenched samples. For excitation of the phycobilisome at 590 nm (Figure 5A), the emission of phycocyanin (650 nm), remained unchanged in quenched cells; however, the emission of allophycocyanin (665 nm), the terminal allophycocyanin emitters (685 nm), and PS I Chl *a* (725 nm) were all decreased. For the excitation of Chl *a* at 435 nm, there were no significant changes in fluorescence emission (Figure 5B).

Excitation spectra for 77 K fluorescence emission at 685 nm (terminal allophycocyanin emitters) are shown for quenched and unquenched M8 mutant cells in Figure 6A. Quenched cells show decreased contributions from components at 570, 620 (phycocyanin), and 650 nm (allophycocyanin). The excitation spectra for the 685 nm emission show no contribution from Chl *a*, consistent with the idea that this emission is predominantly from terminal allophycocyanin emitters in the M8 mutant and not from Chl *a*. The excitation spectra for PSI Chl *a* emission at 725 nm (Figure 6B) do show significant contributions arising from Chl *a* absorption at 435 nm, and there are no changes in the amount of these contributions between quenched and unquenched samples. There are, however, decreases in the peaks at 570, 620, and 650 nm in the quenched samples. These data indicate that energy transfer from the phycobilisome to PSI is reduced in the quenched samples and that Chl *a* is not involved in the quenching mechanism. Thus, an increase in energy transfer from the phycobilisome to PSI cannot be the explanation for the blue-light-induced quenching of phycobilisome emission.

Picosecond time-resolved fluorescence decay data were collected from M8 mutant cells to further explore the origins of blue-light-induced fluorescence quenching. The kinetic decay data were fit with a sum of exponential decay components using a global decay analysis, as described in Materials and Methods. The resulting decay associated spectra (DAS) are shown in Figure 7 for excitation at 407 nm and in Figure 8 for excitation at 650 nm. Both excitation wavelengths required four decay components for the global fit of decay kinetics. The light of 407 nm is absorbed relatively equally by phycocyanin, allophycocyanin, and Chl *a*, whereas the light of 650 nm is absorbed predominantly by allophycocyanin. In dark-adapted cells, for 407 nm excitation, the fastest decay component had a lifetime of about 35 ps and a complex spectrum with a negative amplitude at shorter wavelengths (peak at 660 nm) and a positive amplitude at longer wavelengths (peak at 690 nm). The negative amplitude reflects a fluorescence rise component resulting from an energy transfer from a pigment directly excited by the laser pulse into the observed component. The 660 nm negative peak identifies the origin of this rise component in allophycocyanin, which is thus likely receiving excitation energy from a neighboring shorter wavelength form of phycocyanin on a time scale of about 35 ps. The positive peak at 690 nm in the fast decay component is

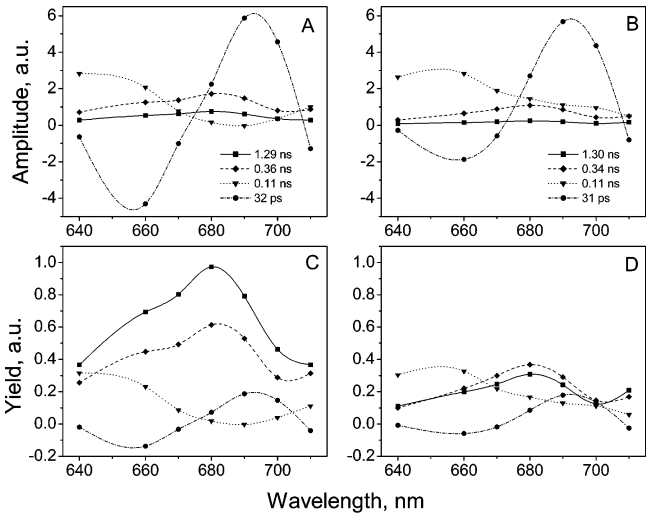

FIGURE 7: Decay associated spectra (DAS) from a global analysis of picosecond fluorescence decay kinetics from (A and C) dark-adapted and (B and D) blue-light-quenched M8 cells. The excitation wavelength is 407 nm. The DAS for decay component amplitudes are shown in the upper panels, and the DAS for decay component yields (amplitude times lifetime) are shown in the lower panels.

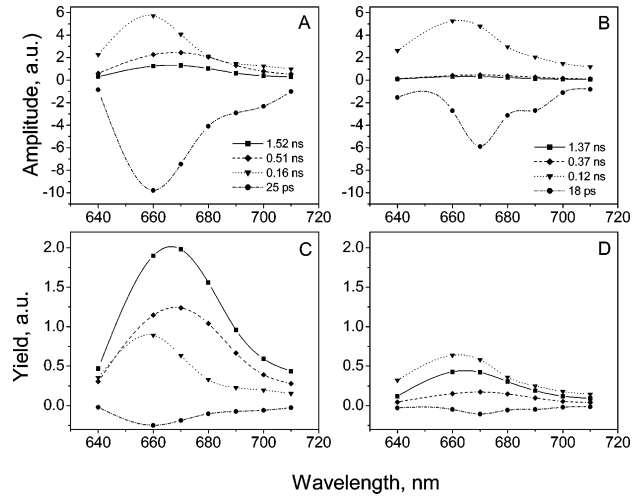

FIGURE 8: Decay associated spectra (DAS) from a global analysis of picosecond fluorescence decay kinetics from (A and C) dark-adapted and (B and D) blue-light-quenched M8 cells. The excitation wavelength is 650 nm. The DAS for decay component amplitudes are shown in the upper panels, and the DAS for decay component yields (amplitude times lifetime) are shown in the lower panels.

typical of PSI decay kinetics and arises from direct excitation of PSI Chl *a* at 407 nm. Quenched cells feature a fast decay component that is very similar to that of dark-adapted cells with one dramatic difference: a significant decrease in the amplitude of the negative peak at 660 nm, which could indicate either a substantial decrease in energy transfer from phycocyanin to allophycocyanin or fast quenching of allophycocyanin excitation. The amplitude of the PSI decay component is identical in quenched and dark-adapted cells. The second fastest decay component has a lifetime of about 110 ps and peaks in the short wavelength region, suggesting an origin in phycocyanin and/or allophycocyanin. The lifetime may reflect energy transfer from these pigments to the terminal phycobilin emitters and/or Chl *a*. There is a significant dip in this component at 680−690 nm in dark-adapted cells, which could arise from the contribution of a fluorescence rise component indicative of energy transfer to terminal emitters. The dip in this decay component disappears in the quenched cells, which is consistent with the quenching of excitation in the phycobilisome. The last two decay components, with lifetimes of about 350 ps and 1.3 ns, have very similar spectral shapes: they both peak at 680 nm and have shoulders at 660 nm. These components likely arise from emission by the terminal phycobilisome emitters. As the 350 ps lifetime is too slow to be indicative of efficient energy transfer from the terminal phycobilin emitters to Chl *a* both decay components probably arise from phycobilisomes uncoupled from the thylakoid membrane. The relative amplitudes of these components are small and decrease further in quenched cells. However, their contribution to overall fluorescence yield (amplitude times lifetime) is high as seen in Figure 7C and D, which show how dramatically they contribute to the blue-light-induced fluorescence quenching. Although the yield of the 350 ps component decreases by approximately 40% in quenched cells, it is the 1.3 ns component that is responsible for most of the loss of fluorescence. The 1.3 ns component is the major contributor to fluorescence yield in dark-adapted cells, and it decreases by 70% in quenched cells.

Figure 8 shows the DAS for both decay amplitudes and yields for excitation at 650 nm, which is predominantly absorbed by allophycocyanin. The decay components are similar to those observed at 407 nm with a few exceptions. The fast decay component now shows only the negative peak at 660 nm and has a shorter lifetime of 25 ps. PSI decay is not observable at this excitation wavelength because of the relatively low absorption by PSI Chl *a* relative to that of allophycocyanin. The negative amplitude of the fast decay component still shows a dramatic decrease in quenched cells. The second fastest decay component is very similar to that observed at 407 nm, although it has a slightly longer lifetime (120−160 ps). Again, this component peaks at 660 nm, reflects decay from phycocyanin and allophycocyanin, and, although the long wavelength dip is not as dramatic as that observed for 407 nm excitation, it is still present in dark-adapted cells but not in quenched cells. The two slower decay components are also similar in shape, lifetime, and behavior to those observed with 407 nm excitation. The longest lived decay component (1.5 ns) again contributes most dramatically to fluorescence quenching.

DISCUSSION

In this work, we have explored the mechanism of blue-light-induced fluorescence quenching in cyanobacteria. This quenching was first described by El Bissati et al. (*17*), who characterized it as a high light effect whose recovery was independent of protein synthesis. Blue-light quenching was later observed in a PSII-less mutant of cyanobacteria by Rakhimberdieva et al. (*18*), who presented a detailed action spectrum for the effect and proposed the involvement of a carotenoid. OCP was subsequently shown to be required for quenching (*21*). We observed similar quenching in our PSII-less M8 mutant; however, the quenching could be observed under considerably lower actinic light levels. The action spectra that we generated for quenching in both the our PSII-less mutant and DCMU-treated wild-type cells were very similar to the spectrum reported by Rakhimberdieva et al. (*18*). This argues for similar origins of quenching in both

mutant and wild-type cells. We further showed that quenching in the wild type and PSII-less mutant had similar kinetics and similar sensitivities to treatment with glutaraldehyde. The protein cross-linker was able to lock in either the quenched or unquenched state in both wild-type and mutant cells, as was freezing the cells at 77 K. The blue-light-induced quenching of fluorescence in cyanobacteria thus involves a reversible change in association and/or conformation of proteins that can be stabilized by cross-linking, or freezing in either the high or the low quenching state. Interestingly, we also showed that quenching in both the wild type and the PSII-less mutant was insensitive to the treatment of cells with 0.5 M phosphate buffer. Such high osmotic buffers have previously been shown to inhibit the mechanism of the light state transition in cyanobacteria (*25*) and to inhibit the apparent mobility of the PBS as determined by fluorescence recovery after photobleaching microscopy (*11*). Clearly, the changes in excitation energy transfer between the PBS, PSII, and PSI associated with the mechanism of the light state transition are independent of those associated with blue-light-induced quenching.

Quenching in Fe-starved wild-type cells was previously shown to be insensitive to 1 M phosphate; however, the phosphate treatment inhibited the dark recovery from quenching (*26*). Our results show that this interesting differential effect of phosphate treatment also occurs in non Fe-starved wild-type cells and the M8 mutant.

As blue-light quenching was so similar in the PSII-less and wild-type cells, we took advantage of the lack of the PSII associated variable Chl *a* fluorescence in the M8 mutant to further investigate the mechanism of blue-light-induced quenching with steady-state and time-resolved fluorescence spectroscopy.

Previous work indicated the origin of quenching to be the PBS core (*21*). Our 77 K fluorescence emission spectra confirm that the light absorbed by Chl *a* is not quenched and clearly show the origin of quenching to be within the PBS. Not all phycobilin components are equally affected by quenching. Upon excitation of phycocyanin, quenching is observed for emission from allophycocyanin, the terminal phycobilin emitters, and PSI but not for emission from phycocyanin itself. Because the pathway of energy transfer through the PBS is from the rods (phycocyanin) to the core (allophycocyanin and the terminal phycobilin emitters), it is clear that the emission spectra are not consistent with phycocyanin as the site of quenching. The decreased emission from allophycocyanin and the terminal phycobilin emitters upon excitation of phycocyanin could arise from a disruption of energy transfer from phycocyanin to allophycocyanin. However, such a disruption would increase the yield of phycocyanin emission as well as its fluorescent lifetime, neither of which was observed. In addition, the excitation spectra for emission from the terminal emitters would be expected to show a decreased contribution from only phycocyanin, but they clearly show decreased contributions from all phycobilin components. The remaining possibility for the selective decrease in fluorescence emission from allophycocyanin and the terminal emitters is the direct quenching of the phycobilisome core. The relative sensitivity of phycocyanin emission yield to quenching in the core will depend on the energetic coupling between the phycobilisome rods and the core. Energy transfer within rods is fast, but energy transfer from rods to cores is relatively slow (~100 ps) and effectively rate limiting (*27*−*29*). A quenching mechanism acting on the cores would thus decrease the excited-state population within the cores to a much greater extent than it would in the rods. Our picosecond fluorescence decay data is consistent with this idea. We found the lifetime of phycocyanin emission to be approximately 110 ps, similar to previous measurements in a PBS/thylakoid membrane preparation (*30*). Consistent with our 77 K steady-state emission spectra, the lifetime and amplitude of this phycocyanin component were effectively independent of quenching. In contrast, a faster (25 ps) allophycocyanin rise component (negative amplitude), also previously observed in PBS/thylakoid preparations (*30*), showed a dramatic decrease in amplitude upon blue-light quenching. This component likely reflects spectral equilibration within the PBS core between short and long wavelength allophycocyanins. The decrease in amplitude would be consistent with a mechanism of blue-light-induced quenching that acted at the PBS core to effectively decrease the excited-state population. This explanation is also consistent with the concomitant large decrease in amplitude of the two terminal emitter decay components (350−500 ps and 1.2−1.5 ns) in quenched cells. Most of the observed fluorescence quenching was due to a decrease in the amplitude of the long (1.3 ns) decay component. This indicates that the majority of quenching originates from a small fraction of uncoupled phycobilisomes.

A mutant cyanobacteria, lacking the water soluble carotenoid binding protein (OCP) has been shown to be incapable of blue-light-induced quenching (*21*). Our action spectra in both the M8 mutant and wild-type cells are also consistent with a carotenoid sensor. Wilson et al. (*21*) proposed a working hypothesis that quenching may be explained by the interaction of the OCP with the core of the phycobilisome. Our cross-linking data supports the idea that blue-light quenching involves changes in protein−protein interactions. The X-ray structure of OCP indicates that the carotenoid in the dark-adapted protein is in the trans configuration (*20*), which is consistent with the UV peak in the action spectra for quenching shown by Rakhimberdieva et al. (*18*) and in this work. We suggest that the absorption of blue light by OCP may cause the trans−cis isomerization of the carotenoid, which triggers a conformational change of the protein. Such a conformational change could allow OCP to trigger changes in the association of other quenching proteins with the phycobilisomes or facilitate interaction of the OCP with the PBS core itself. This latter mechanism could result in quenching by direct energy transfer from the S_1 excited state of allophycyanin to the carotenoid, where the energy would be quickly dissipated as heat.

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