Rapid Electron Transfer to Photosystem I and Unusual Spectral Features of Cytochrome c_6 in *Synechococcus* sp. PCC 7002 in Vivo[†]

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ABSTRACT: Cytochrome c_6 donates electrons to photosystem I (PS I) in *Synechococcus* sp. PCC 7002. In this work, we provide evidence for rapid electron transfer ($t_{1/2} = 3\ \mu s$) from cytochrome c_6 to PS I in this cyanobacterium in vivo, indicating prefixation of the reduced donor protein to the photosystem. We have investigated the cytochrome c_6−PS I interaction by laser flash-induced spectroscopy of intact and broken cells and by redox titrations of membrane and supernatant fractions. Redox studies revealed the expected membrane-bound cytochrome *f*, b_6, and b_{559} species and two soluble cytochromes with α-band absorption peaks of 551 and 553 nm and midpoint potentials of −100 and 370 mV, respectively. The characteristics and the symmetrical α-band spectrum of the latter correspond to typical cyanobacterial cytochrome c_6 proteins. Rapid oxidation of cytochrome c_6 by PS I in vivo results in a unique, asymmetric oxidation spectrum, which differs significantly from the spectra obtained for cytochrome c_6 in solution. The basis for the unusual cytochrome c_6 spectrum and possible mechanisms of cytochrome c_6 fixation to PS I are discussed. The occurrence of rapid electron transfer to PS I in cyanobacteria suggests that this mechanism evolved before the endosymbiotic origin of chloroplasts. Its selective advantage may lie in protection against photo-oxidative damage as shown for *Chlamydomonas*.

Oxygenic photosynthesis involves two photosystems (PS II[1] and PS I), the cytochrome $b_6 f$ complex as a proton pump, and mobile electron shuttles that interconnect these membrane-bound protein complexes. Electron transfer from the cytochrome $b_6 f$ complex to photosystem I (PS I) typically occurs via a soluble electron transfer protein, either cytochrome c_6 or plastocyanin (reviewed in ref *1*). Higher plants use an acidic plastocyanin exclusively for this reaction, whereas some algae use either plastocyanin or cytochrome c_6 depending on Cu availability. Many cyanobacteria use only cytochrome c_6 which may be either acidic or basic, and some have the ability to produce a corresponding acidic or basic plastocyanin (*2*). The distribution of plastocyanin has not been systematically investigated among cyanobacteria, but it occurs in rather diverse members of this group, such as the filamentous *Anabaena* and unicellular *Synechocystis* strains (*3*) and the chlorophyll *a*/*b*-containing *Prochlorothrix hollandica* (*4*). Chloroplasts originated early within the cyanobacterial lineage (*5*), presumably from cyanobacteria capable of expressing an acidic plastocyanin.

The electron transfer reaction from plastocyanin or cytochrome c_6 to the primary donor (P700) of PS I has been investigated in detail in several eukaryotes. In vitro, a substantial fraction (∼30−40%) of flash-oxidized P700 is rapidly reduced with first-order kinetics in PS I particles from higher plants ($t_{1/2} = 12\ \mu s$) (*6*) and algae ($t_{1/2} = 3\ \mu s$) (*7*). Laser flash studies of intact chloroplasts (*6*, *8*) and cells of the alga *Chlorella* (*9*) show fast reduction of more than 90% of the photo-oxidized P700 centers. The fast electron transfer is consistent with reduced plastocyanin or cytochrome c_6 fixed to the PS I reaction center prior to flash excitation. Transient complex formation between the soluble electron donor and the PS I protein complex depends on the PsaF (*7*, *10*) and secondarily the PsaJ (*11*) subunits of PS I. Mutational analysis of PsaF in *Chlamydomonas reinhardtii* identified several conserved lysine residues near the N-terminus that are important for rapid $P700^+$ reduction (*12*, *13*). These lumen-exposed residues are thought to interact electrostatically with an acidic patch in plastocyanin or cytochrome c_6. Deletion of psaF still allows photosynthetic growth of *Chlamydomonas* but considerably slows electron transfer to $P700^+$ (*10*).

The crucial N-terminal, lysine-containing domain of PsaF is missing in cyanobacteria (*12*, *14*). In vitro, in the cyanobacteria *Synechocystis* PCC 6803 (*15*), *Synechococcus elongatus* (*16*), and *Anabaena* PCC 7119 (with plastocyanin as the electron donor) (*17*), the reduction of PS I is ∼2 orders of magnitude slower than in algae and higher plants.

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[1] Abbreviations: FCCP, carbonyl cyanide 4-[(trifluoromethoxy)-phenyl]hydrazone; PS II, photosystem II; PS I, photosystem I; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; MOPS, 3-(*N*-morpholino)-propanesulfonic acid.

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Consistent with these data, $P700^+$ reduction in vivo by cytochrome c_6 or plastocyanin in *Synechocystis* has a half-time of 150−200 μs (*18*), and removal of the PsaF and PsaJ subunits in *Synechocystis* 6803 has no impact on electron transfer kinetics in vitro (*19*). However, rapid reduction ($t_{1/2}$ = 4 μs) of *Anabaena* PCC 7119 PS I by the basic *Anabaena* plastocyanin has been observed in vitro (*17*), and there are reports in the early literature of rapid ($t_{1/2}$ = 10−40 μs) electron transfer to PS I in vivo in *Plectonema boryanum* (*20*) and a thermophilic *Synechococcus* sp. (*21, 22*). These observations indicated that at least certain cyanobacteria have evolved rapid electron transfer to PS I and that different types of donor−acceptor interactions may exist.

To better elucidate electron transfer to PS I in cyanobacteria, we have undertaken a detailed characterization of in vivo electron transfer from an acidic cytochrome c_6 protein to PS I in *Synechococcus* sp. PCC 7002, a rapidly growing mesophilic cyanobacterium widely used for genetic analysis (*23*). We report here the occurrence of rapid ($t_{1/2}$ = 3 μs) electron transfer from cytochrome c_6 to the PS I reaction center and a unique distortion of the time-resolved cytochrome c_6 spectrum in *Synechococcus* cells.

MATERIALS AND METHODS

Culture Conditions of Synechococcus. Cells were cultivated in medium A as described in ref *24*. Cultures for experiments were grown in 500 mL of medium A in flat-sided culture vessels, bubbled with 5% CO_2 in air, and incubated under cool-white fluorescent lamps at approximately 30 °C. The rate of gas flow and light intensity were increased during culture growth up to a light intensity of ca. 40 000 lux during the final 12 h of culture. Cells were harvested during mid to late log phase.

Preparation of Membrane and Supernatant Fractions. All steps were performed at 4 °C. For membrane preparations, cells were centrifuged at 5000*g* for 15 min and resuspended in 50 mM MOPS buffer (pH 7). Cells were broken with a French press at 20 000 psi and again centrifuged for 10 min at 6000*g* to pellet unbroken cells. The supernatant was centrifuged for 1 h at 200000*g* to pellet all membranes. These were resuspended in 50 mM MOPS buffer (pH 7). Soluble proteins of the original supernatant fraction were concentrated by ultrafiltration in a Centricon 10 unit (10 000 molecular weight cutoff, Amicon).

Electrochemical Redox Titrations. Redox titrations were performed in an electrochemical cell as described in ref *25*. The gold grid, which was used as a working electrode, was modified with pyridine 3-carboxaldehydethiosemicarbazone to avoid irreversible protein adhesion. Titrations were performed with the spectrophotometer described in refs *26* and *27* in the spectrum mode. The cell was filled with the concentrated supernatant from cell lysates in 50 mM MOPS (pH 7) and 50 mM KCl. For redox titrations of membrane fragments, the pellet was resuspended in 1 volume of 50 mM MOPS buffer (pH 7) and 50 mM KCl in the presence of 13 redox mediators (Table 1) at a concentration of 100 μM each. Titrations were performed over the potential range from 450 to −500 mV in steps of 25 mV in the range of cytochrome redox reactions and 50 mV outside of this range. The midpoint potential of the hemes and their corresponding spectra were calculated either by the program mEh-fit (*28*) in a global fit procedure (Levenberg−Marquart algorithm and general least-squares algorithm) on the data obtained from electrochemical redox titrations or by a fit of a sum of Nernst curves to data values at single wavelengths.

Table 1: Redox Mediators for Electrochemical Titrations[a]

mediator	midpoint potential (mV)
ferricyanide	430
p-benzoquinone	280
2,5-dimethyl-2-benzoquinone	180
1,2-naphthoquinone	145
phenazine methyl sulfate	80
1,4-naphthoquinone	60
menadione	0
2,5-dihydroxy-2-benzoquinone	−60
anthraquinone	−100
2-hydroxy-1,4-naphthoquinone	−145
anthraquinone 1,5-disulfonate	−170
anthraquinone 2-sulfonate	−225
benzyl viologen	−359

[a] Redox mediators were used at a concentration of 100 μM each in electrochemical redox titrations on membrane fragments of *Synechococcus*. The midpoint potentials of mediators are given for pH 7.

Kinetic Measurements. Kinetic experiments were performed with a home-built flash spectrophotometer (*29*), where the absorption changes are sampled at discrete times by short flashes. These flashes are provided by a Nd:Yag-pumped (355 nm) optical parametric oscillator which produces monochromatic flashes [1 nm full width at half-maximum (fwhm) with a duration of of 6 ns]. Excitation was provided by a dye laser pumped by the second harmonic of a Nd:Yag laser (700 nm, 10 mJ). Continuous illumination was provided by a laser diode (690 nm, 30 mW). Experiments were performed in either 10 mM NaCl, 10 mM $NaHCO_3$, 5 mM Hepes buffer (pH 7.5) (for experiments on intact cells) or 50 mM MOPS buffer (pH 7) (for all other experiments). For experiments on intact cells, cells were maintained under argon in the presence of 10 μM DCMU and 1 mM NH_4OH to eliminate flash-induced absorption changes from PS II (*30*). Unless stated otherwise, the ionophore FCCP (10 μM) was added to disrupt the transmembrane electrochemical potential.

Kinetic data were measured at several wavelengths between 380 and 430 nm and between 540 and 570 nm. Data were fitted by the global fit procedure, "mexfit" [Levenberg−Marquardt algorithm and a general least-squares algorithm (*31*)], to obtain the rate constants and spectra of the different kinetic phases. The residual of the fit thereby is the remaining absorption difference between the initial state before flash excitation and the final state reached after completion of the fitted kinetic phases.

RESULTS

Equilibrium Redox Titrations. Figure 1 shows the results of equilibrium redox titrations of membrane (panel C) and supernatant fractions (panels A and B) from *Synechococcus* cells. Two cytochromes could be detected in the supernatant (Figure 1A,B). One shows a Soret band maximum at 417 nm, an α-band maximum at 553 nm, and a midpoint potential of ∼370 mV, which are characteristic of cytochrome c_6 (*32*). A second cytochrome was detected with a Soret band peak at 417 nm, an α-band peak at 551 nm, and a midpoint potential of −100 mV. Titration of the membranes revealed

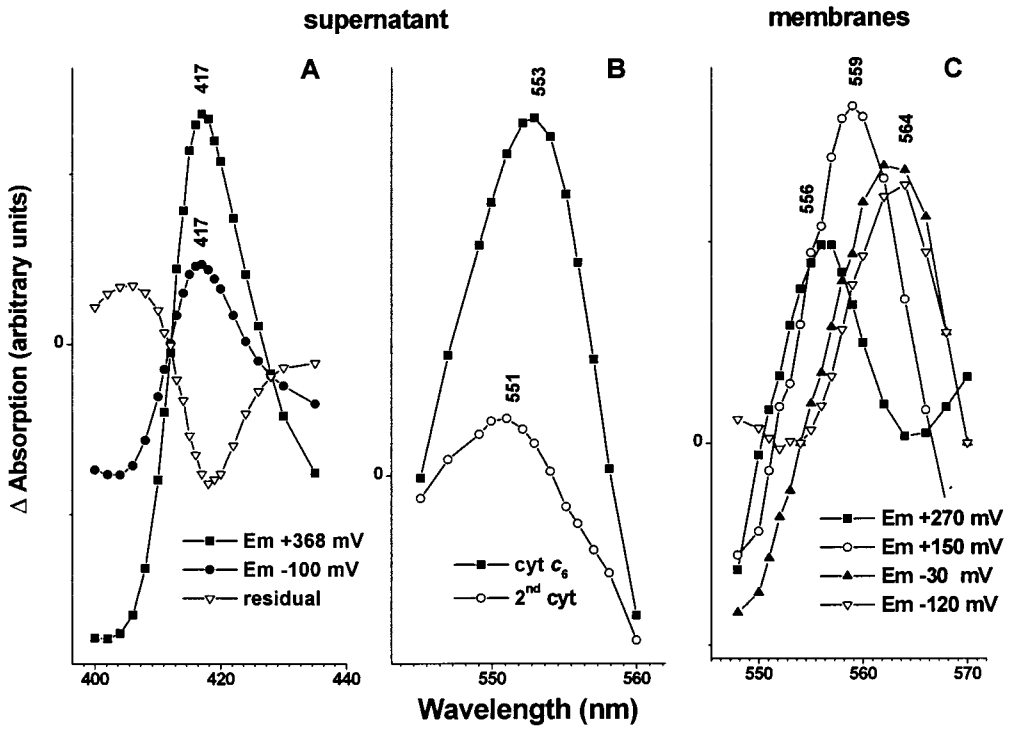

FIGURE 1: Reduced minus oxidized difference spectra showing the results of redox titrations of supernatant (A and B) and membrane fractions (C) of *Synechcooccus*. Membrane titrations were performed in the presence of the redox mediators listed in Table 1. Difference spectra and midpoint potentials were obtained either by a global fit procedure (described in Materials and Methods, panels A and B) or by a fit of a sum of Nernst functions to the data at different wavelengths and subsequent calculation of difference spectra. The residuals of the fit in panel A can be explained by the loss of the sample from a leaky titration cell during the experiment.

cytochrome α-band peaks at 556, 559, 562, and 564 nm (Figure 1C). These were assigned to cytochrome *f* (270 mV), cytochrome b_{559} (150 mV), cytochrome b_H (−30 mV), and cytochrome b_L (−120 mV), respectively. The data for cytochrome b_{559} could be further deconvoluted to show two redox potentials of 130 and 220 mV, of which the low-potential form was predominant (ca. 80%, data not shown). Spectra in the Soret band spectral region could not be recorded from membranes because of a high background of chlorophyll absorption. Fujita et al. (*33*) report a PS II/cytochrome $b_6 f$ ratio of 1.2 in high-light-grown *Synechocystis* 6714. Our results with *Synechococcus* sp. PCC 7002, shown in Figure 1C, are consistent with this since we observed similar amplitudes for the four cytochromes. If one heme b_{559} per PS II is assumed, the cytochrome $b_6 f$ complex and PS II were in nearly equal proportions in the membranes. Unfortunately, it was not possible to compare cytochrome concentrations in membranes and the supernatant under our experimental conditions.

In Vivo Electron Transfer Kinetics. Figure 2 shows the kinetics of electron transfer in *Synechococcus* cells after laser flash excitation resulting in a single turnover of PS I. Kinetics were recorded at 554 nm, a wavelength close to the α-band maximum of cytochrome c_6, the physiological donor of electrons to the PS I reaction center. The data are best fitted with two components: a fast phase with a half-time of 3 μs (70%) and a slower phase with a half-time of 25 μs (30%). We assign the 3 μs phase to $P700^+$ reduction by cytochrome c_6 prefixed to the PS I reaction center prior to the actinic flash.

The reactions in the millisecond time range should reflect cytochrome c_6 re-reduction and the reactions of the $b_6 f$ complex. These kinetics are sensitive to flash intensity and to the addition of FCCP, an uncoupler known to collapse the transmembrane potential. Addition of FCCP and low flash intensities both accelerate the reduction kinetics. Reduction kinetics after a saturating actinic flash in the

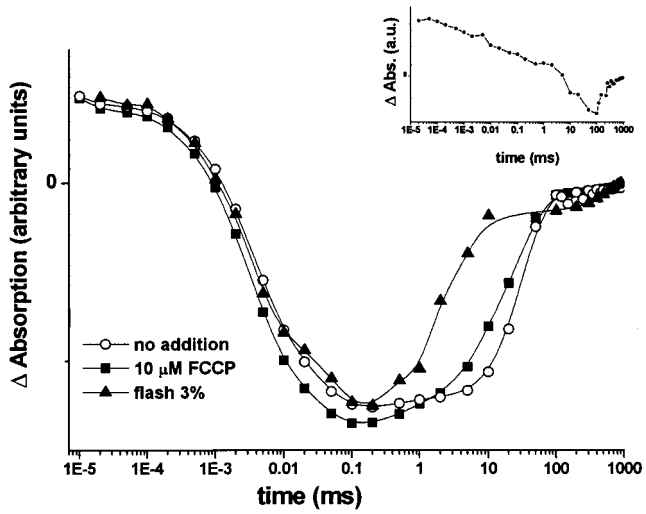

FIGURE 2: Flash-induced kinetics of intact *Synechococcus* cells and mixed membrane and supernatant fractions (inset). Data for intact cells were collected at 554 nm from cells suspended in buffer containing 10 μM DCMU and 1 mM NH_4OH. Kinetics were normalized to equal amounts of oxidized P700 formed, measured by the absorption difference 10 ns after the actinic flash. Data are from cells exposed to saturating actinic flashes in the absence (○) and presence of 10 μM FCCP (■) and to flashes at 3% saturation in the presence of 10 μM FCCP (▲). The inset represents data collected at 552 nm from a mixture of *Synechococcus* membrane and supernatant fractions after exposure to a saturating actinic flash.

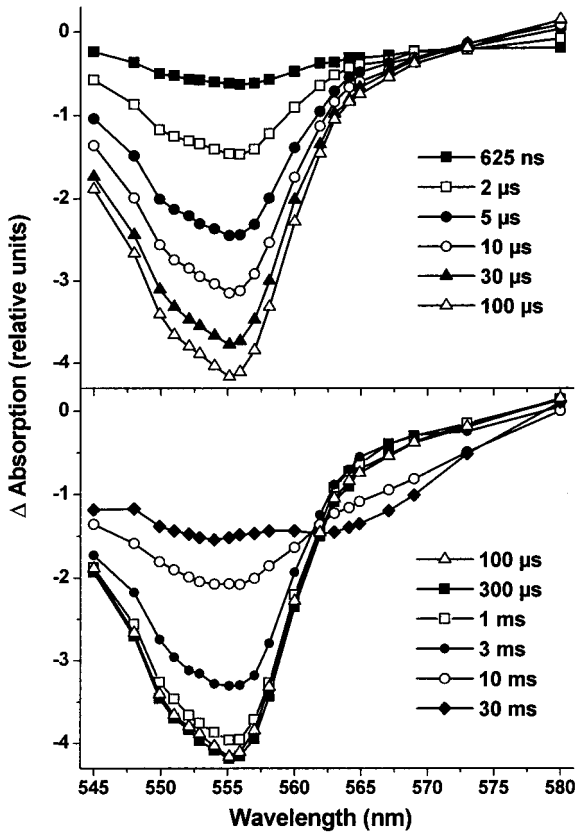

FIGURE 3: Time-resolved spectra of flash-induced absorbance changes in the cytochrome α-band region of intact *Synechococcus* cells. Spectra were reconstructed from kinetics data collected at single wavelengths (such as those shown in Figure 2). The top panel shows spectra of the oxidation phase of cytochrome c_6, and the bottom panel shows spectra mostly of the reduction phase.

presence of the uncoupler FCCP [Figure 2 (■)] can be fitted by a sum of two exponentials with half-times of 1 (10%) and 22 ms (90%). The latter phase was further accelerated (to a $t_{1/2}$ of 7 ms), when the flash intensity was lowered to 3% of saturation [Figure 2 (▽)]. Flash saturation was assessed from the signal amplitude measured 10 ns after the flash, which is proportional to the amount of oxidized P700. Acceleration in the presence of FCCP suggests that transmembrane charge transfer reactions (i.e., the turnover of the cytochrome b_6f complex) are involved in the reduction of cytochrome c_6. Acceleration of the reduction kinetics at low flash intensity is due either to multiple turnovers of the b_6f complex (expected under saturating conditions if the b_6f/PS I ratio is less than 1) or to slower kinetics of this complex because of the higher flash-induced transmembrane potential at saturating flash intensities. The rapid cytochrome c_6 oxidation reactions were not influenced by either the addition of FCCP or lower flash intensity.

Spectra of Redox Components Involved in Flash-Induced Electron Transfer Reactions in Vivo. To investigate in greater detail the rapid in vivo electron transfer from cytochrome c_6 to the PS I reaction center, flash-induced spectra of the cytochrome α-band region were obtained from whole cells of *Synechococcus* (Figure 3). An asymmetric cytochrome oxidation spectrum begins to appear as early as 625 ns after the flash. Over the whole time scale that was investigated, the spectra remained asymmetric and thus differ significantly from those obtained from equilibrium redox titrations in solution (Figure 1B; see Figure 5 for comparison).

Figure 4A shows the deconvolution of kinetics in the α-band region at saturating flash intensities. The spectra of the components with $t_{1/2}$ values of 3 and 25 μs peak at 554 nm with a shoulder at 550 nm. The shoulder is less pronounced but still present in the 25 μs spectrum (Figure 5). The major part of the reduction kinetics at 554 nm can be described by a half-time of 22 ms (see Figure 2). The spectrum of this phase is shown in Figure 4A as the residual of the fit. The reaction kinetics were investigated further by recording additional spectra in the Soret and α-band spectral region at different flash intensities. Figure 4B shows the deconvolution of kinetics in the α-band region obtained with a weak actinic flash (3% of the intensity of a saturating flash). In this experiment, the first data were measured 20 μs after the actinc flash so that the fast cytochrome c_6 oxidation was not resolved. The spectrum of the absorption changes measured at 20 μs shows the asymmetric cytochrome spectrum with a maximum at 554 nm and a shoulder at 550 nm. Three kinetic components were needed to fit the data. A phase with a half-time of 70 μs and a spectrum with a trough at 556 nm indicate the oxidation of cytochrome *f* (■). The component with a half-time of 1 ms (○) and a spectrum displaying two peaks (556 and 566 nm) can be interpreted as reflecting the reduction of cytochrome *f* (peak at 556 nm) and cytochromes b_6 (peak at 566 nm). Finally, a half-time of 7 ms and a peak at 566 nm characterize the oxidation of the *b* hemes (◇). The spectra and half-times of the cytochrome b_6f reactions are similar to those obtained for *C. reinhardtii* in vivo with subsaturating flashes (*34*). It is noteworthy that the spectrum of the oxidation of cytochrome *f* is much smaller in amplitude than the spectrum of its reduction phase. This is not surprising since cytochrome *f* is oxidized at the expense of the oxidized cytochrome c_6 so that the spectrum of this electron transfer reaction is expected to result from the difference between the spectra of the reduced cytochrome *f* and cytochrome c_6. These two spectra strongly overlap (see Figure 1), with a slightly higher extinction coefficient for cytochrome *f* across the whole spectral range (*35*), and the resulting absorption changes therefore are expected to be small.

In the Soret band region (Figure 4C), half-times of 3 and 110 μs can be fitted to the data. They correspond reasonably to the half-times obtained for the rapid oxidation of cytochrome c_6 and oxidation of cytochrome *f* in kinetic experiments in the α-band spectral region (Figures 2 and 4A,B). The spectrum of the 3 μs component peaks at 417 nm and therefore supports the assignment of this component to cytochrome c_6 oxidation. As stated previously, we were unable to perform the redox titration of the membrane fragments in the Soret band region because of a strong chlorophyll absorption background so that the oxidation spectrum of cytochrome *f* in *Synechococcus* is not documented in this spectral region. However, spectra of the isolated cytochrome b_6f complex from the cyanobacterium *Synechocystis* sp. PCC 6803 (D. Bald, M. Roegner, and F. Baymann, unpublished results) show a peak at 422 nm comparable to the spectrum observed here for the 110 μs component.

In summary, we observed the rapidly appearing, asymmetric cytochrome oxidation spectrum in the α-band spectral

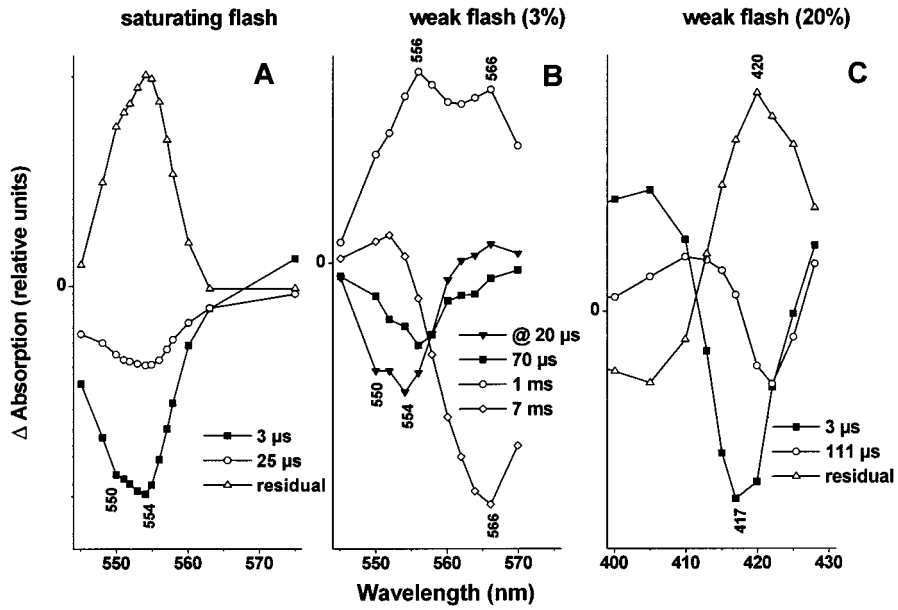

FIGURE 4: Deconvolutions of flash-induced kinetics data obtained at different wavelengths and flash intensities from *Synechococcus* cells. The spectra shown were obtained from data deconvoluted by the global fit algorithm described in Materials and Methods. The half-times of appearance of the calculated spectra after the flash are indicated. Data were collected with saturating (A) or weak flashes (B and C) in the α-band (A and B) and Soret band spectral regions (C). The 20 μs spectrum in panel B is the first spectrum measured in this experiment and not the result of the curve fit. The curves labeled "residual" correspond to the residual of the fit (i.e., the remaining absorption difference between the initial state before flash excitation and the final state reached after completion of the fitted kinetic phases).

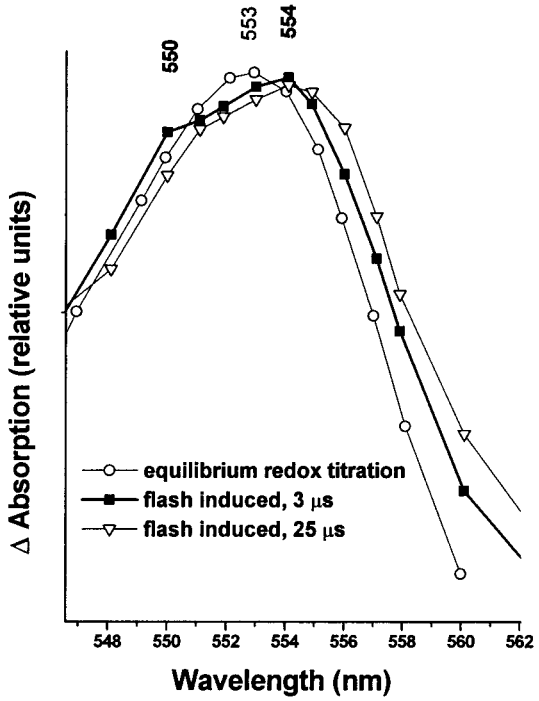

FIGURE 5: Comparison of cytochrome c_6 spectra obtained from equilibrium redox titrations and from spectral deconvolution of kinetics data obtained by saturating flashes. Spectra were normalized to approximately the same amplitudes.

region. Because of its rapid appearance and persistence under all experimental conditions [i.e., high- and low-light cultures (data not shown), saturating and weak flashes], the asymmetric spectrum appears to represent a single cytochrome species. The asymmetric spectral features may be induced by formation of a complex of reduced cytochrome c_6 and PS I or by the specific environment of cytochrome c_6 in the thylakoid lumen of *Synechococcus* cells as discussed below.

Data from the weak flash experiments in the Soret and in the α-band spectral region are consistent with subsequent re-reduction of this species by cytochrome *f* of the cytochrome $b_6 f$ complex.

Electron Transfer Reactions in a Mixture of Membrane and Supernatant Fractions. To understand the influence of the cellular environment on the spectral changes of cytochrome c_6, we investigated flash-induced electron transfer reactions in a mixture of membrane and supernatant fractions from *Synechococcus*. The inset in Figure 2 shows the kinetics at 552 nm after flash excitation. Oxidation of cytochrome c_6 by the oxidized photosystem I reaction center ($P700^+$) occurred in the millisecond time range. The corresponding spectrum measured 100 ms after the actinic flash has an α-band peak at ~552 nm (data not shown), comparable to the spectrum obtained in equilibrium redox titrations. Its maximal amplitude, compared to that of $P700^+$ formation, is approximately 20% of the amplitude obtained in vivo, indicating that, in the present experiment, less cytochrome c_6 was present per PS I than in the cell. The slow electron transfer kinetics indicate that under these conditions formation of a complex of cytochrome c_6 and PS I does not takes place.

DISCUSSION

Rapid Cytochrome c_6 Oxidation in Synechococcus. Our finding of rapid ($t_{1/2}$ = 3 μs) electron transfer from cytochrome c_6 to PS I substantiates earlier reports of rapid ($t_{1/2}$ = 10−40 μs) $P700^+$ reduction in cyanobacteria in vivo (*20*−*22*) and in vitro (*17*, *36*) and suggests that fast electron transfer to PS I may be much more common in cyanobacteria than previously believed. The overall charge (P_I value) of cyanobacterial cytochrome c_6 proteins is either basic or acidic. Of the cyanobacteria found to display rapid PS I reduction, *P. boryanum* (P_I = 9.32) (*20*), *Anabaena* PCC 7119 (P_I = 9.25) (*17*), and *Pseudanabaena* PCC 6903 (P_I

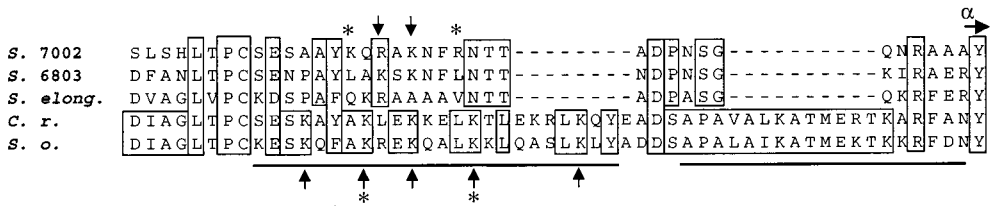

FIGURE 6: Alignment of the lumenal, N-terminal domains of mature PsaF proteins from cyanobacteria and chloroplasts. The thick arrow (α) marks the approximate start of the first transmembrane helix. Boxes mark residues conserved in cyanobacteria or chloroplasts. Arrows under the plastid sequences mark conserved lysines that are important for plastocyanin or cytochrome c_6 binding, and asterisks mark the critical K16 and K23 residues (*13*). Arrows above the *Synechococcus* PCC 7002 sequence mark conserved positively charged residues, and asterisks mark positive charges near the critical K16 and K23 positions of plastids. Sequences of *Synechocystis* PCC 6803 (*S.* 6803), *S. elongatus* (*S. elong.*), *C. reinhardtii* (*C. r.*), and *Spinacia oleracia* (*S. o.*) are from GenBank, and that for *Synechococcus* PCC 7002 (*S.* 7002) is from ref *60.*

= 8.0) (*36*) have basic cytochrome c_6 proteins. The relative charge of cytochrome c_6 in the thermophilic *Synechococcus* sp. used in refs *21* and *22* is not known but is probably acidic as in *S. elongatus* (pI 4.8) (*37*), and *Synechococcus* sp. PCC 7002 used here (pI 4.89, C. Nomura and D. Bryant, personal communication). There are currently no reports of rapid $P700^+$ reduction by plastocyanin in cyanobacteria. We conclude that cyanobacteria probably evolved rapid electron transfer to PS I before the endosymbiotic origin of chloroplasts (perhaps more than once, from both acidic and basic cytochrome c_6 donors) and that this reaction is not an exclusively eukaryotic invention as suggested previously (*10*, *14*).

Mechanism of Rapid P700 Reduction in Cyanobacteria. Electron transfer reactions of the rate and extent reported here require physical proximity of adequate concentrations of donor and acceptor centers (*38*). For PS I reduction by cytochrome c_6 and plastocyanin, interaction is believed to occur first via electrostatic attractions followed by a rearrangement which fixes the reduced donor center close to P700 in the dark (*1*, *17*). Upon flash oxidation, rapid electron transfer occurs from the prefixed plastocyanin or cytochrome c_6 to $P700^+$. A series of elegant studies with the green alga *Chlamydomonas* have shown that both plastocyanin and cytochrome c_6 donor−PS I reaction complexes require the PsaF subunit of photosystem I (*7*, *10*, *12*). Of specific importance is an N-terminal, lumen-exposed domain of PsaF which contains several conserved Lys residues (*12*, *13*). A model has been proposed in which a negative patch on plastocyanin binds to the N-terminal, exposed Lys of PsaF and then rotates closer to the P700 reaction center, becoming fixed to the PS I core via hydrophobic interactions (*12*). Cytochrome c_6 possesses a corresponding negative domain. In addition, a hydrophobic region close to the heme edge and two conserved residues within it, an arginine and a phenylalanine/tryptophan, are thought to interact with PS I (*14*, *39*, *40*).

The lack of the N-terminal Lys-containing domain of PsaF proteins has been cited as the reason for the apparent absence of rapid P700 reduction in cyanobacteria (*10*, *14*). Indeed, the elimination of PsaF had no impact on electron transfer to PS I in vitro in the thermophile *S. elongatus* (*16*) or in *Synechocystis* PCC 6803 (*19*). However, data presented here for *Synechococcus* sp. PCC 7002, which possesses the "less efficient," cyanobacterial-style, PsaF protein, clearly demonstrate rapid electron transfer to PS I in this cyanobacterium in vivo. The lumen-exposed N-terminal domain of cyanobacterial PsaF proteins is ~18 residues shorter than that of PsaF from chloroplasts (Figure 6). Nevertheless, cyanobacterial PsaF proteins possess several positively charged residues in the N-terminal region that could interact with the acidic cytochrome c_6 protein. Two different alignments have been presented (*12*, *14*). We show a slightly modified scheme in Figure 6 which allows greater conservation in the N-terminal region. For example, a positive charge (R22) is conserved in *Synechococcus* near the key K23 position of chloroplast PsaF proteins. A mutation in this position (K23Q) in *Chlamydomonas* dramatically affects plastocyanin cross-linking to PS I and lowers the second-order rate constants of electron transfer from plastocyanin and cytochrome c_6 to PS I (*13*). Perhaps significantly, the PsaF proteins of *Synechocystis* 6803 and *S. elongatus*, which lack the fast electron transfer reaction, lack a positive residue near position 23. Alternatively, binding of cytochrome c_6 to PS I may occur primarily via interactions with the hydrophobic core of PS I. Interactions with hydrophobic domains of the core PsaA and PsaB subunits (*41*, *42*) or the PsaN subunit (*43*) of PS I have already been proposed. Mutations of the basic *Anabaena* 7119 cytochrome c_6, in which positive charges are expected to interact with PS I, show that a patch of positive residues and a hydrophobic region near the heme edge are important for efficient binding to PS I, but not necessarily via PsaF, in vitro (*44*). Finally, a high concentration of the donor protein in the restricted lumenal space in vivo may result in prefixation of cytochrome c_6 even if the electrostatic and hydrophobic interactions between PS I and cytochrome c_6 are less pronounced in cyanobacteria than in chloroplasts. In line with this reasoning, Drepper has observed for PS I and cytochrome c_6 from *S. elongatus* rapid electron transfer in ~1.5 μs in vitro but only at very high concentrations of cytochrome c_6 (K_d = 1 mM, F. Drepper, personal communication). Of note also is the finding of Soriano et al. (*45*) that the *Chlamydomonas* cytochrome *f* lysine residues required for efficient docking to plastocyanin in vitro are much less important for efficient electron transfer in vivo.

Selective Advantage of Rapid Electron Transfer to PS I. At first glance, rapid P700 reduction seems unnecessary. The rate-limiting step in photosynthetic electron transport is plastoquinol oxidation by the cytochrome b_6f complex (*46*) with a half-time of approximately 10 ms in *Synechococcus* sp. PCC 7002 (see the kinetics discussion above, and ref *47*). PsaF deficient *Chlamydomonas* grow photoautotrophically at rates comparable to that of the wild type (*10*). However, Hippler et al. (*48*) have recently found that *Chlamydomonas* lacking the PsaF subunit or carrying the

PsaF K23Q mutation, which slows $P700^+$ reduction, are very sensitive to high light intensity (>400 μE m^{-2} s^{-1}) under aerobic conditions. Overabsorption of light energy in the absence of a productive sink (reduced P700) may lead to triplet chlorophyll formation which in turn can react with O_2 to produce damaging singlet O_2 (*49*). We note, however, that in both the absence and presence of an efficient docking site for plastocyanin, the electron transfer to $P700^+$ is significantly faster than the rate-limiting step of the photosynthetic electron transfer chain (3 or 200 μs vs 10 ms). Thus, under illumination conditions where the photon flux per PS I is more than one photon every 10 ms, accumulation of $P700^+$ is expected irrespective of fast electron transfer from plastocyanin (or cytochrome c_6). Although questions remain about the mechanism of protection, it is perhaps significant that fast electron transfer to PS I in cyanobacteria has been detected only in planktonic species where intermittent exposures to high irradiance may be expected.

Unusual Asymmetric Cytochrome c_6 Spectrum from Synechococcus Cells. Time-resolved spectra of rapid cytochrome oxidation from *Synechococcus* cells exhibited an α-band maximum at 554 nm and a prominent shoulder at 550 nm (Figures 3 and 5). These spectra differ considerably from those obtained by equilibrium redox titration of the supernatant, which showed a symmetrical α-band peak at 553 nm (Figures 1 and 5) typical of cyanobacterial cytochrome c_6 (*2*, *32*).

We believe the flash-induced spectra from *Synechococcus* cells represent the oxidation of a single cytochrome species. The asymmetric spectrum appears rapidly ($t_{1/2} = 3$ μs) after photo-oxidation of P700, and the relative amplitudes of the 554 and 550 nm peaks remained proportional under different growth and experimental conditions (low and high light, weak and saturating flashes, presence or absence of FCCP). If two or more components (cytochrome c_6, cytochrome *f*, cytochrome M, or another low-potential cytochrome) were involved, the spectral characteristics and the rapid reaction kinetics could only be explained by supercomplex formation in constant stoichiometries under all conditions. We consider this unlikely. Apart from cytochrome c_6, we detected only one soluble cytochrome ($E_m = -100$ mV, α-band peak at 551 nm, Figure 1) in *Synechococcus* PCC 7002. Its midpoint potential is between those of cytochrome M ($E_m = 150$ mV, α-band peak at 550 nm) of *Synechocystis* PCC 6803 (*50*−*52*) and a low-potential cytochrome c_{449} ($E_m = -260$ mV) first reported for *Anacystis nidulans* (now *Synechococcus* PCC 6301) (*53*, *54*). The relationship of cytochrome c_{551} of *Synechococcus* PCC 7002 to these cytochromes and to a partially characterized cytochrome c_{552} (cytochrome M?) of *Synechococcus* PCC 6301 (*53*, *54*) is not yet clear. Because of their low midpoint potentials, these cytochromes would not be efficiently reduced by the cytochrome $b_6 f$ complex, and their participation in light-induced photosynthetic reactions seems highly improbable (*52*).

The α-band absorption of a heme arises from the two electronic transitions in the heme plane. The energy of these transitions and consequently the wavelength of the respective absorption bands are identical as long as the intrinsic high symmetry of the heme is not perturbed. The influence of the heme environment on the electron distribution in the macrocycle ring can change the energy and the occurrence of one transition with respect to the other, leading to an asymmetric or even split α-band. There are precedents. Recently, Trp 4 adjacent to the heme face of cytochrome *f* from *Phormidium* was seen to cause the observed asymmetric and longer wavelength (556 nm) α-band of cyanobacterial relative to chloroplast cytochrome *f* species (*55*). At cryogenic temperatures, reduced occupancy of vibrational states results in smaller spectral line widths, and nearby heme transitions are therefore distinguished. A split α-band was thus reported for cytochrome c_6 from *Scenedesmus* (*56*) and *Synechocystis* 6803 (*57*) at 77 K.

An asymmetric α-band of cytochrome c_6 of *Synechococcus* PCC 7002 was observed here by flash-induced cytochrome oxidation in vivo but not in redox-induced difference spectra in vitro. The asymmetric α-band may therefore be induced either by modification of the heme environment in the very restricted intrathylakoidal (lumenal) space in vivo or by interaction of the reduced cytochrome c_6 with PS I prior to flash excitation. In the first case, a high ionic strength, a high concentration of a particular ion, or the binding of a protein or small molecule might be imagined and should induce the asymmetric spectrum during oxidation as well as reduction of the protein. We observed asymmetric spectra over the whole time range that was investigated. In the ≥70 μs time range, however, absorbance changes induced by redox reactions of the cytochrome $b_6 f$ complex can be superimposed with the spectral changes of cytochrome c_6, as shown by the deconvolution in Figure 4. Because cytochrome c_6 is reduced at the expense of cytochrome *f*, their respective spectral contributions cannot be easily separated kinetically. In vivo cytochrome reduction spectra showing a 550 nm shoulder and appearing with millisecond kinetics ($t_{1/2} \sim 10$ ms) have been reported previously for *Synechocystis* PCC 6803 (*58*, *59*) and *Synechococcus* PCC 7002 (*47*) following long flash (100 ms) oxidation of P700. These spectra, however, could be attributed to contributions from cytochrome *f* (α-band peak at ∼556 nm), cytochrome c_6 (α-band peak at ∼553 nm), and possibly a high-potential *c*-type cytochrome (α-band peak at ∼550 nm) like cytochrome c_M (*50*).

If the interaction of cytochrome c_6 and PS I induces the spectral change, we would expect the oxidation of cytochrome c_6 but not its reduction by the cytochrome $b_6 f$ complex to provide an asymmetric spectrum. Further experimental evidence will be needed to determine the basis for the cytochrome c_6 α-band asymmetry. Studies are underway with a PsaF-less mutant of *Synechococcus* to obtain such data.

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