Inactivation of the petE Gene for Plastocyanin Lowers Photosynthetic Capacity and Exacerbates Chilling-Induced Photoinhibition in the Cyanobacterium Synechococcus

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We describe the identification and expression of a petE gene in Synechococcus sp. PCC 7942, a cyanobacterium previously thought to lack plastocyanin. The petE gene is a 420-bp open reading frame that encodes a protein 70 to 75% similar to plastocyanins from other cyanobacteria. Synechococcus possesses a single genomic copy of petE located immediately upstream of the clpB gene. It is transcribed as a single mRNA (550 bases) and, in contrast to most other photobionts, the level of petE expression in Synechococcus is unaffected by variable copper concentrations during acclimated growth. Inactivation of petE does not prevent photoautotrophic growth, but does induce a dramatic increase in mRNA for the alternative electron carrier cytochrome c₆. Despite this adjustment, loss of plastocyanin results in slower growth, lower photosystem I content, and a decreased maximum capacity for photosynthetic electron transport. The mutant is also more susceptible to chilling-induced photoinhibition during a shift from 37 to 25°C, at which temperature its inherently lower photosynthetic capacity exacerbates the normal slowing of electron transfer reactions at low temperatures. Under similar conditions, the amount of petE message in the wild type decreases by 50% in the 1st h, but then increases dramatically to almost three times the 37°C level by 9 h.

Plastocyanin is a small, Cu-binding polypeptide that functions in oxygenic photosynthesis as an electron carrier between the Cyt b₆f complex and PSI in chloroplasts of algae and higher plants, and also in many cyanobacterial strains. Plastocyanin transfers single electrons via the reversible oxidation and reduction of its active Cu center (Ho and Krogmann, 1984). The apoprotein is typically encoded by a single-copy gene, designated petE. In plants, the nuclear-encoded pre-plastocyanin protein contains a two-domain, or bipartate, transit peptide that directs it first through the chloroplast envelope and then into the lumen of the thylakoid membrane, where the transit peptide is cleaved off by a specific peptidase (de Boer and Weisbeek, 1991). In addition to guiding translocation, the transit peptide also appears to prevent premature association of the Cu cofactor with the plastocyanin apoprotein prior to sub-organellar targeting. Once in the lumen, the Cu-containing plastocyanin is extremely stable (Li and Merchant, 1992). In chloroplasts of higher plants, plastocyanin is the sole mobile carrier within the thylakoid lumen that catalyzes electron transfer from the reduced heme in Cyt f within the b₆f complex to the oxidized reaction center Chl of PSI, P700⁺ (Boulter et al., 1977). Many cyanobacteria and eukaryotic algae, however, also contain at least one other alternative electron carrier, of which the best studied is Cyt c₆.

Cyt c₆, formerly known as Cyt c₅₅₂ or c₅₅₃, is a small, water-soluble, iron-heme protein similar to the mobile c₆-type Cyts in anoxygenic photosynthetic bacteria (Dickerson, 1980). In some cyanobacteria and algae it has a function equivalent to plastocyanin in higher plants, mediating electron flow between the Cyt b₆f complex and PSI. As for the Cu center of plastocyanin, the bound iron-heme of Cyt c₆ also transfers electrons via reversible oxidation/reduction. Most photobionts with Cyt c₆ also possess plastocyanin, with both redox proteins having the potential for mediating photosynthetic electron transport (Krinner et al., 1982). Certain cyanobacterial strains, however, apparently have only Cyt c₆ to transfer electrons from the Cyt b₆f complex to PSI (Sandmann and Böger, 1980).

In most species of algae and cyanobacteria that possess both plastocyanin and Cyt c₆, the proportion of the two proteins is strongly regulated by the Cu concentration in the growth medium (Ho and Krogmann, 1984; Merchant and Bogorad, 1986a; Bovy et al., 1992; Nakamura et al., 1992; Zhang et al., 1992; Ghassemian et al., 1994). Cyt c₆ is produced when insufficient Cu is available for plastocyanin synthesis (Wood, 1978; Sandmann et al., 1983), whereas little or no transcripts for Cyt c₆ are detected in the presence of Cu, indicating transcriptional or posttranscriptional control (Merchant and Bogorad, 1986a). In Cu-replete medium, plastocyanin presumably serves as the preferential electron donor, since the synthesis of the alternative Cyt is strongly repressed. It is when Cu is absent that the heme-containing Cyt c₆ is induced to compensate for plastocyanin, enabling photosynthetic electron transport to continue. Regulation of plastocyanin by Cu, however, appears to differ among different organisms. In the green microalga Chlamydomonas reinhardtii, for example, expression of the petE gene is unaffected by Cu concentration, but apoplas-tocyanin is rapidly degraded (Merchant and Bogorad, 1986b; Li and Merchant, 1992). In contrast, plastocyanin synthesis is transcriptionally or posttranscriptionally regu-

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Abbreviations: Chl, chlorophyll; Km, kanamycin.
lated by Cu in other algal strains such as Scenedesmus, and in several strains of cyanobacteria such as Synechocystis sp. PCC 6803 (Van der Plas et al., 1989; Li and Merchant, 1992; Nakamura et al., 1992; Zhang et al., 1992).

The presence of alternative electron carriers in certain algae and cyanobacteria accounts for the continued photosynthetic growth of strains in which plastocyanin or Cyt c₆ have been genetically inactivated. A similar degree of interchageability is believed to exist between the different mobile carriers in photosynthetic bacteria, as exemplified by the functional substitution of isocytochrome c₅ for the normal Cyt c₅ during transfer of electrons from the Cyt bc₁ complex to reaction centers (Fitch et al., 1989). To date, inactivation mutants in algae and cyanobacteria lacking either plastocyanin or Cyt c₆ show no significant disruption in electron transfer reactions or in their capacity for photoautotrophic growth (Merchant and Bogorad, 1987b; Lauenbach et al., 1990; Zhang et al., 1994). This is true even if the particular null mutant is grown under conditions that repress the expression of the other redox protein (Zhang et al., 1994). There is now substantial evidence that Cyt c₆ also functions as an electron donor to the respiratory Cyt c oxidase complex in some cyanobacteria (Alpes et al., 1984; Binder et al., 1984; Scherer et al., 1988), presumably due to the presence of both respiratory and photosynthetic protein complexes within the thylakoid membranes.

The unicellular Synechococcus sp. PCC 7942 has long been considered one of the cyanobacterial strains lacking plastocyanin, supposedly possessing Cyt c₆ as its sole electron carrier for reducing P700⁺ (Aitken, 1976; Rippka et al., 1979; Aoki et al., 1983; Sandmann, 1986; Van der Plas et al., 1989; Geerts et al., 1994). We now report the discovery of an actively expressed petE gene in Synechococcus sp. PCC 7942 that codes for a protein homologous to plastocyanin in other cyanobacteria. In contrast to most photobionts, expression of the Synechococcus sp. PCC 7942 petE gene is not influenced by changes in Cu concentrations within the growth medium. Disruption of this gene also produces distinct phenotypic changes within the mutant, the most significant being a decrease in photosynthetic capacity and an increased susceptibility to photoinhibition induced by low temperature.

**MATERIALS AND METHODS**

**Culture Conditions**

Each strain of the cyanobacterium Synechococcus sp. PCC 7942 was grown in BG-11 inorganic medium (Rippka et al., 1979) buffered with 10 mM 3-(N-morpholino) propane sulfonic acid (pH 7.5) at 37°C under a continuous irradiance of 50 μmol photons m⁻² s⁻¹ in batch cultures (Clarke et al., 1995). When required, Cu-limited growth conditions were obtained by omitting CuSO₄ from the medium, whereas for high-Cu conditions, the CuSO₄ concentration was increased to 1 μM. The ΔpetE strain was maintained on solid BG-11 plates and in liquid precultures with 5 μg mL⁻¹ of the antibiotic Km to maintain selection. For experimental batch cultures, however, ΔpetE was grown without Km to eliminate the possibility of antibiotic-induced changes in phenotype. All cultures were bubbled with 5% CO₂ in air to avoid changes in antennae size due to low inorganic carbon concentrations. Cells in the exponential-growth phase with a Chl concentration of 2 to 3 μg mL⁻¹ were used for all experiments.

**Cloning and Sequencing of petE**

The petE gene from Synechococcus was identified after DNA sequencing upstream of the clpB gene, which is described elsewhere (Eriksson and Clarke, 1996). Dideoxy sequencing of each clone was carried out using a thermal cycle amplification system (Promega). Analysis of DNA and protein sequences was carried out using the PileUp program in the University of Wisconsin Genetics Computer Group package (Devereux et al., 1984).

**DNA Hybridization**

Southern blot analysis was performed as described by Clarke et al. (1994) using total DNA (5 μg) completely digested with one of several restriction enzymes. Restriction fragments were separated on a 1% agarose gel, transferred to a nylon filter (Hybond-N, Amersham), and then UV cross-linked according to the manufacturer’s recommendations. Filters were hybridized to a radioactive, homologous, gene-specific DNA probe at 65°C in 6X SSC, 5X Denhardt’s solution, 0.2% SDS, 2 μm EDTA, and 100 μg mL⁻¹ denatured herring sperm DNA. The PCR-amplified probes were radiolabeled using the random priming method (Feinberg and Volgelstein, 1983) with [α-32P]dCTP, and then purified through a 1-mL Sephadex G-50 column. After hybridization, filters were washed in 0.1X SSC, 0.1% SDS at 65°C and then exposed to x-ray film (Cronex, DuPont).

**RNA Hybridization**

Total RNA was isolated from Synechococcus sp. PCC 7942 according to the method described by Campbell et al. (1995). Northern blot analysis was performed with RNA samples (5 μg) denatured by glyoxylation and fractionated in 1% agarose gels in 10 mM NaPO₄ (pH 7.0). RNA was transferred to a nylon filter via capillary blotting, baked at 80°C for 30 min, and then cross-linked under UV light for 2 min. Prior to hybridization, filters were washed in 10 mM Tris-HCl (pH 8.0) for 5 min at 65°C to deghostxylate the RNA. Filters were hybridized to radiolabeled petE or cytA probes for 16 h at 65°C in 6X SSC, 5X Denhardt’s solution, 0.5% SDS, and 100 μg mL⁻¹ denatured herring sperm DNA. Probes were radiolabeled and purified as described for DNA hybridizations. Following hybridization, filters were washed twice at 65°C for 15 min in 1X SSC, 0.1% SDS and then once at 65°C for 30 min in 0.1X SSC, 0.1% SDS. The level of each transcript was measured directly from the autoradiograph (Bio-Rad) and quantified relative to the amount of 16S RNA (Kulkarni et al., 1992).
**Results**

**Characterization of the petE Gene in Synechococcus**

**Construction of petE Inactivation Plasmid and Transformation**

The petE inactivation plasmid was constructed by first synthesizing two specific oligonucleotides (with EcoRI restriction sites) and then PCR-amplifying from the positive genomic clone a DNA fragment containing the complete petE open reading frame plus 300 to 400 bp on either end. The fragment was then ligated into the EcoRI site of the plasmid pUC9 and transformed into *Escherichia coli* DH5α cells. The resulting plasmid construct was linearized with SphI, which cut within the N terminus of petE, and was then blunt-ended using T4 DNA polymerase. Into this site was ligated a *HincII*-cut 1.3-kb DNA fragment containing the gene coding for Km resistance. The construct was transformed into competent *E. coli* DH5α cells, positive transformants were selected on medium containing Km, and plasmids containing the correct inactivation construct were verified by restriction endonuclease digests. *Synechococcus* sp. PCC 7942 cells were then transformed with the linearized construct according to Van der Plas et al. (1990). Putative transformants were selected on BG-11 plates supplemented with Km (5 μg mL⁻¹).

**Photosynthetic Measurements and Chilling Treatments**

Chl a fluorescence yield and net oxygen evolution were measured simultaneously using a system of cuvette, magnetic stirrer, oxygen electrode, and actinic lamp (Hansatech, King’s Lynn, UK), compatible with a pulse-amplitude-modulated Chl fluorometer (Walz, Effeltrich, Germany), described in detail by Clarke et al. (1995) and Campbell et al. (1996). A range of actinic light similar in quality to the growth light was used for measurements. Cells were not CO₂-limited during measurements. Photochemical and nonphotochemical quenching of PSII variable fluorescence were calculated according to the method of van Kooten and Snel (1990), along with the relative efficiency of excitation-energy capture by PSII reaction centers and the apparent yield of PSII photochemistry (Genty et al., 1989).

The cuvette system described above was used to measure oxygen evolution resulting from a train of saturating, 2.5-μs, single-turnover flashes of white light supplied at a frequency of 10 Hz. The resulting rate of oxygen evolution was converted to PSII content by assuming that every active PSII center produces one O₂ molecule for every four flashes (Myers et al., 1980; Park et al., 1995; Campbell et al., 1996). PSII to PSII ratios were derived from this value by assuming 52 Chls/PSII and 118 Chls/PSI, as determined for the very closely related strain *Synechococcus* sp. PCC 6301 under a variety of conditions (Myers et al., 1980).

Chilling treatments were performed as described by Campbell et al. (1995). Wild-type and ΔpetE cultures were shifted from 37 to 25°C at control growth conditions under constant light. Photosynthetic performance and pigment content were monitored over the 9-h treatment. Samples were also taken of the wild-type culture for detection of petE transcripts.

**petE Is a Single-Copy Gene**

To determine the number of petE copies in the *Synechococcus* genome, total DNA was isolated and cut with several different endonucleases selected from the known DNA sequence for petE. The restricted DNA fragments were then hybridized under high stringency with a specific 200-bp petE probe (Fig. 2A). The probe hybridized to only one DNA fragment for each restriction endonuclease except DNA cut with SphI, in which two fragments were detected (Fig. 2B). Comparison of the predicted number of restriction fragments with the pattern from the Southern blot confirmed that petE is a single-copy gene in *Synechococcus* sp. PCC 7942, a finding consistent with data from petE genes in other cyanobacteria (Van der Plas et al., 1989; Briggs et al., 1990; Ghassemian et al., 1994).

**Expression of petE**

Because of the ambiguity concerning the existence of plastocyanin in *Synechococcus* sp. PCC 7942, we first deter-
mained whether the petE gene is expressed under our standard conditions of steady-state growth in BG-11 medium at 37°C, 50 µmol photons m⁻² s⁻¹, and 5% CO₂ in air. As shown in Figure 3, the petE gene in Synechococcus is constitutively expressed as a monocistronic message of 550 bp. The length of this transcript is somewhat shorter than those of other cyanobacteria (700-800 bp), mainly because of the deletions within the central and C-terminal domains. We next analyzed the level of petE expression during growth with different concentrations of Cu, since changes in Cu availability from 0 to 1 µM strongly influence plastocyanin synthesis in most cyanobacteria and plants. Synechococcus cultures were grown in a BG-11 medium lacking Cu, in standard BG-11 medium containing 0.3 µM Cu, or in BG-11 medium supplemented to a final concentration of 1 µM. In cells at equivalent stages of growth, the changes in Cu concentration consistently had no significant effect on the level of petE expression (Fig. 3).

**Inactivation of petE**

To study the importance of plastocyanin for photosynthetic electron transport in Synechococcus sp. PCC 7942, we inactivated the petE gene by disrupting the coding region via insertion of an antibiotic-resistance gene. This first entailed the preparation of a plasmid construct in which a 1.3-kb Km-resistance cassette was inserted into the Sppl site situated in the 5' half of the petE gene (Fig. 4A). After verification by restriction endonuclease digests, the petE inactivation construct was excised from the plasmid and the linear DNA fragment was transformed into actively growing, wild-type Synechococcus sp. PCC 7942. Transformants were then selected for their ability to grow on BG-11 medium supplemented with Km.

To confirm the correct integration of the petE inactivation construct and its complete segregation within the mutant genome, Southern hybridization was performed using total DNA isolated from both the mutant (ΔpetE) and the wild type. For this analysis, a second DNA probe was prepared, which spanned most of the petE gene and the region down-

**Figure 1.** Alignment of Synechococcus sp. PCC 7942 plastocyanin with other known cyanobacterial homologs. Alignments were made using the PileUp program, with conserved amino acids boxed and gaps shown as dots. The putative processing site of the transit peptide is indicated by the arrowhead, and the putative Cu ligands are shown by asterisks above the sequence. Complete names of each cyanobacterium and its corresponding plastocyanin accession number are: Anabaena sp. PCC 7937, X14342; Nostoc sp. PCC 7120, L19417; Phormidium laminosum, X73207; Synechococcus sp. PCC 7942, U20147; Synechocystis sp. PCC 6803, X54105; and Prochlorothrix hollandica, U13912.

**Figure 2.** Single genomic copy of petE gene in Synechococcus sp. PCC 7942. A, Restriction map of the petE gene in Synechococcus indicating the position of relevant endonuclease sites, as well as the location and length of the DNA probe complementary to petE. B, Southern blot of total DNA from Synechococcus digested with endonucleases Spol, EcoRI, BamHI, HindIII, PstI, SacI, Scal, and KpnI and hybridized to the petE-specific DNA probe. DNA standard-size markers are shown at the left.
Characterization of the \( \text{petE} \) Gene in \textit{Synechococcus}

Reduced Photosynthetic Capacity

Previous studies with cyanobacteria reported no significant phenotypic changes in photosynthetic activity or growth characteristics resulting from the inactivation of \( \text{petE} \) gene expression (Zhang et al., 1994). In contrast, the \( \Delta \text{petE} \) strain from \textit{Synechococcus} sp. PCC 7942 exhibited several phenotypic changes relative to the wild type under standard growth conditions. When grown at 50 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) at 37°C with 5% \( \text{CO}_2 \), the generation time of \( \Delta \text{petE} \) was slightly slower than that of the wild type (Table I). The mutant also had a consistently lower PSI-to-PSII ratio, which is reflected in a slight increase in the ratio of phycocyanin to Chl. There were no significant changes in dark-respiration rates or in photosynthetic oxygen evolution activity under nonsaturating, growth-light conditions (i.e. 50 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)).

To probe the photosynthetic characteristics of the mutant more closely, we compared the light responses of oxygen evolution for both the \( \Delta \text{petE} \) and the wild-type strains to determine their capacity for photosynthetic electron transport and the efficiency of PSII photochemistry or quantum yield (Fig. 5). The quantum yield of PSII, as indicated from the initial slope of each light-response curve, was identical for both strains. The mutant’s maximum capacity for electron transport, however, was significantly reduced relative to the wild type, as shown by lower light-saturated rates of

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**Figure 3.** Cu-independent expression of \( \text{petE} \) in \textit{Synechococcus} sp. PCC 7942. Wild-type cells were grown under standard conditions in BG-11 medium with the \( \text{CuSO}_4 \) constituent either unchanged (normal, 0.3 \( \mu \text{M} \)), absent (low), or supplemented (high, 1 \( \mu \text{M} \)). RNA was isolated from cells at equivalent stages of exponential growth, and the \( \text{petE} \) transcript was detected by hybridization with the DNA probe described in Figure 2. The figure shows a representative autoradiogram from one of two replicates. Molecular size markers in kilobases are indicated on the left.

**Figure 4.** Insertional inactivation of the \( \text{petE} \) gene. The \( \Delta \text{petE} \) construct was made by inserting the gene coding \( \text{Km} \) resistance into the \( \text{SphI} \) site of the original construct altered this restriction motif, a single \( \text{SphI} \) fragment is predicted for the correct \( \text{ApefE} \). Furthermore, the insertion of the \( \text{Km} \) cassette introduced two additional \( \text{PstI} \) sites, resulting in the extra \( \text{PstI} \) fragment detected in the mutant. The lack of a wild-type hybridization profile in \( \Delta \text{petE} \), even after prolonged exposures, confirmed that complete segregation had occurred. Afterward, the \( \text{petE} \) probe was removed and the filter was reprobed with the 1.3-kb \( \text{Km} \)-resistance cassette (Fig. 4C). This probe strongly annealed to the expected 1.3-kb \( \text{PstI} \) fragment in \( \Delta \text{petE} \), but failed to give any signal with the wild type, confirming the presence of the \( \text{Km} \) cassette in the mutant strain. The hybridization results were verified by PCR amplification using two oligonucleotides specific for the \( \text{petE} \) gene separated by 200 bp, a region in \( \Delta \text{petE} \) that is disrupted by the insertion of the \( \text{Km} \) cassette. The expected 200- and 1500-bp fragments were amplified from the wild type and mutant, respectively, reconfirming the successful formation and segregation of the \( \Delta \text{petE} \) strain (data not shown).

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oxygen evolution activity (Fig. 5). The Chl fluorescence characteristics of both strains also showed that the photochemical efficiency of PSII was unaffected by the loss of plastocyanin (Fig. 6A). The level of nonphotochemical quenching, however, was considerably higher in ΔpetE at all irradiances, indicating that the mutant is shifted toward state II, with more excitation energy transferred from the phycobilisome antennae to PSI (Fig. 6B). Cells are driven toward state II by reduction of the electron-transport chain from oxygen evolution or fluorescence has been previously observed in different species of cyanobacteria (Campbell, 1996; Campbell and Oquist, 1996), and is in contrast to the strong correlation between the two parameters in higher plants (Krause and Weis, 1991).

Changes in cytA Gene Expression

Since the loss of plastocyanin reduced maximum photosynthetic capacity in Synechococcus sp. PCC 7942, but did not prevent photoautotrophic growth, we investigated whether compensatory changes in the level of the alternative electron carrier Cyt c6 had occurred in the mutant. As observed in an earlier study, cytA is a low-level transcript in Synechococcus sp. PCC 7942 and is particularly unstable, detectable only as a weak, diffuse signal from 200 to 1200 bases (Laudenbach et al., 1990). Using a similar cytA-specific probe, we also observed a similar weak, diffuse signal in the wild type when grown at 50 μmol photons m⁻² s⁻¹ at 37°C (Fig. 7). In the ΔpetE strain, however, the amount of cytA mRNA was several times higher, indicating a compensatory increase in cytA expression in response to the inactivation of petE (Fig. 7).

Low-Temperature Shift

We next tested whether the limitation in photosynthetic electron transport in the mutant would exacerbate the chilling-induced photoinhibition that occurs in Synechococcus sp. PCC 7942 when shifted from 37 to 25°C. As shown in Figure 8A, the inactivation of oxygen evolution was considerably more severe in the mutant than in the wild type. After 2 h at 25°C, the mutant retained less than 10% of the control photosynthetic activity measured prior to the shift, whereas the wild type retained around 50%. For the following 7 h, the level of oxygen evolution in ΔpetE remained below 20% of the control value, whereas the wild type maintained 40 to 50% activity (Fig. 8A). Despite the more extensive loss in overall electron transport, the drop in PSII photochemical efficiency in the mutant was similar to that observed in the wild type (Fig. 8B). The loss of plastocyanin, therefore, has little effect on PSII efficiency per se, but increases the extent of chilling-induced photoinhibition, presumably by restricting electron-transfer reactions farther down the transport chain.

In addition to photosynthetic measurements, the level of petE transcript was followed in the wild type throughout the shift from 37 to 25°C (Fig. 9). After an initial decrease of around 50%, the amount of petE transcript steadily increased throughout the low-temperature treatment, reaching nearly three times the level of the 37°C control.

Table 1. Comparison of physiological characteristics in the Synechococcus sp. PCC 7942 wild-type and ΔpetE strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Wild Type</th>
<th>ΔpetE</th>
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<tr>
<td>Generation time (h)</td>
<td>7.7 ± 0.1</td>
<td>8.3 ± 0.5</td>
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<tr>
<td>Acclimated net O₂ evolution (μmol O₂ mg⁻¹ Chl h⁻¹)</td>
<td>96 ± 17</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>Maximum net O₂ evolution (μmol O₂ mg⁻¹ Chl h⁻¹)</td>
<td>330 ± 8</td>
<td>274 ± 18</td>
</tr>
<tr>
<td>Dark respiration (μmol O₂ mg⁻¹ Chl h⁻¹)</td>
<td>−61 ± 10</td>
<td>−74 ± 17</td>
</tr>
<tr>
<td>Phycocyanin/Chl (A₆₂₅/A₆₇₈)</td>
<td>0.83 ± 0.02</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>PSI/PSII</td>
<td>3.3 ± 0.5</td>
<td>2.7 ± 0.2</td>
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All values were measured under growth conditions of 50 μmol photons m⁻² s⁻¹, 37°C, and bubbling with 5% CO₂, except maximum O₂ evolution, which was measured under saturating light of 290 μmol photons m⁻² s⁻¹. Averages of determinations on three to five independent cultures, ± se, are shown.
Characterization of the petE Gene in Synechococcus

Figure 6. Light-response curves of PSII fluorescence parameters. The Chl fluorescence characteristics for the wild-type (■) and ΔpetE (○) strains were measured at irradiances from 0 to 290 μmol photons m⁻² s⁻¹. Cells of both strains were grown under identical temperature, light, and CO₂ conditions and used at similar stages of exponential growth (approximately 2 μg Chl ml⁻¹). A, Proportion of open PSII reaction centers (i.e., photochemical quenching, qP). B, Nonphotochemical quenching (qN). C, Photochemical yield of PSII electron transport (ΦPS II). Where indicated, values represent averages ± SE (n = 3–5).

DISCUSSION

We have described in this study the cloning and sequencing of a petE gene coding for plastocyanin from Synechococcus sp. PCC 7942, a widely studied strain of unicellular cyanobacteria that until now had been thought to lack this photosynthetic electron carrier. The predicted plastocyanin from Synechococcus has all the structural characteristics of other cyanobacterial homologs, including a recognizable transit peptide for translocation into the thylakoid lumen and the conserved residues involved in Cu binding. As in other cyanobacterial strains, the Synechococcus plastocyanin does not contain the negatively charged patch of amino acids between residues 40 and 65 common to eukaryotic homologs (Redinbo et al., 1994). These small domains of negatively charged amino acids are thought to mediate the interaction of plastocyanin with Cyt f and P700 from PSI (Beoku-Betts et al., 1983). Synechococcus also lacks the hydrophobic patch conserved in algae and higher plants surrounding the Cu ligand His-87, a domain possibly also involved with PSI interaction (Nordling et al., 1991).

Early observations by Visser et al. (1974) detected electron paramagnetic resonance signals within intact Synechococcus cells, which were indicative of Cu-containing plastocyanin. Later studies, however, failed to detect this redox protein and concluded that Synechococcus sp. PCC 7942 was one of those cyanobacteria, like Nostoc muscorum and Spirulina platensis, that lacked plastocyanin. This conclusion was based initially on the failure of polyclonal antibodies directed against plastocyanin from spinach to cross-react with a homologous protein from Synechococcus sp. PCC 7942 (Aitken, 1976), a finding reaffirmed in another study using antibodies against the plastocyanin from Anabaena sp. PCC 7937 (Geerts et al., 1994). The absence of plastocyanin in Synechococcus was also inferred using optical spectroscopy (Aoki et al., 1983) and heterologous gene hybridization (Van der Plas et al., 1989). Instead, other proteins such as Cyt c₆ were proposed as alternative mobile carriers mediating electron transport between the Cyt b₅ complex and PSI. In hindsight, it is not surprising that these methods were unsuccessful in detecting plastocyanin from Synechococcus sp. PCC 7942, given the low level of sequence conservation between the different genes and polypeptides. Indeed, this was exemplified in our own studies by the failure of antibodies directed to more closely related homologs (i.e. Chlamydomonas and Phormidium) to detect the Synechococcus plastocyanin.

Figure 7. Increased level of cytA expression in ΔpetE. RNA was isolated from wild-type (wt) and ΔpetE cells at equivalent stages of exponential growth, and the cytA transcript for Cyt c₆ was detected by hybridization with a DNA probe specific for the entire gene. The figure shows a representative autoradiogram from one of two replicates. Molecular size markers are indicated on the left.
basic and acidic proteins, respectively (Davies et al., 1980; Bovy et al., 1992; Zhang et al., 1992; Varley et al., 1995). In Bogorad, 1986a; Sandmann, 1986; Van der Plas et al., 1989; cytA petE of expression in many green algae and other and cytA expression concomitantly decreases, being essentially undetectable after 60 min (Bovy et al., 1992). Synthesis of plastocyanin in Anabaena occurs with as little as 0.05 μM Cu, but reaches a maximum at around 0.6 μM (Sandmann and Boger, 1980; Bovy et al., 1992). Despite the absence of such regulation, the Cu-independent expression of petE in Synechococcus sp. PCC 7942 is consistent with that of cytA expression in this strain, which is also Cu-insensitive (Laudenbach et al., 1990). It is interesting that this common regulatory feature of the petE and cytA genes in Synechococcus is also consistent with the more general theory that plastocyanin and Cyt c₆ from a given organism have co-evolved (Ho and Krogmann, 1984), a proposal originally based on the matching pi for these two evolutionarily unrelated proteins.

Apart from Synechococcus sp. PCC 7942, Cu-independent expression of petE has been reported only in the green alga Chlamydomonas (Merchant and Bogorad, 1986b). In Chlamydomonas, petE expression is constitutive, whereas the accumulation of plastocyanin is dependent on the presence of Cu because of the highly unstable nature of the apoprotein

Within a single photosynthetic species, plastocyanin and Cyt c₆ often have similar pi values (Ho and Krogmann, 1982, 1984). Plastocyanin in all eukaryotic photobionts and Cyt c₆ in certain algae are acidic proteins with pi values between 4 and 6 (Ho and Krogmann, 1982). In cyanobacteria, filamentous and unicellular strains typically have basic and acidic proteins, respectively (Davies et al., 1980; Ho and Krogmann, 1984; Briggs et al., 1990; Arudchandran et al., 1994). This pattern holds true for the unicellular Synechococcus sp. PCC 7942, with the mature plastocyanin having a predicted pi of 4.6, whereas that of the Cyt c₆ protein is around 6.5 (Laudenbach et al., 1990). The functional significance of differently charged plastocyanin proteins in multi- and unicellular cyanobacteria has yet to be ascertained.

As for other cyanobacteria, the petE gene in Synechococcus sp. PCC 7942 is a single-copy gene constitutively transcribed as a monocistronic message. Its expression is not affected by the Cu concentration in the growth medium, which is in marked contrast to the strong regulation by Cu of petE and cytA expression in many green algae and other cyanobacteria (Ho and Krogmann, 1984; Merchant and Bogorad, 1986a; Sandmann, 1986; Van der Plas et al., 1989; Bovy et al., 1992; Zhang et al., 1992; Varley et al., 1995). In the cyanobacterium Anabaena, plastocyanin mRNA is detectable at the 37°C control (100%).

Figure 8. Chilling-induced photoinhibition following a shift from 37 to 25°C. Wild-type (■) and Apeff (○) cultures growing at 37°C (0-h control) were shifted to 25°C for 9 h. A, Net oxygen evolution under the growth-light intensity of 50 μmol photons m⁻² s⁻¹. B, Photochemical efficiency of PSII reaction centers (Fₗ/Fₘ). Results are expressed as a percentage of the 37°C control values (100% oxygen evolution = 95–115 μmol O₂ mg⁻¹ Chl h⁻¹; 100% Fₗ/Fₘ = 0.50–0.52). Values represent averages from two replicates.

Figure 9. Changes in petE expression following a shift from 37 to 25°C. Wild-type cells growing at 37°C (0-h control) were shifted to 25°C for 9 h, and samples were taken at regular intervals for RNA isolation. A, The petE mRNA was detected by hybridization using the 200-bp probe previously described in Figure 2. Molecular size markers in kilobases are indicated on the left. B, The level of petE transcript was later quantified relative to the amount of 16S rRNA, which was measured in Atm units at the 37°C control (100%).
within the thylakoid lumen (Merchant and Bogorad, 1986b: Li and Merchant, 1995). It is possible that plastocyanin in Synechococcus sp. PCC 7942 is also regulated by Cu at the posttranslational level rather than the more common transcriptional, posttranscriptional controls on petE expression. Arguing against this proposal is the differential effect of Cu on cytA expression in these two photobionts. Transcription of cytA is strongly repressed by Cu in Chlamydomonas (Merchant and Bogorad, 1987a), whereas it remains unaffected in Synechococcus sp. PCC 7942 (Laudenbach et al., 1990). Given the usually close relationship between plastocyanin and Cyt c6, it is likely that plastocyanin content in Synechococcus sp. PCC 7942 is also relatively insensitive to Cu concentration as long as sufficient Cu is available for plastocyanin function.

Inactivation of petE expression in Synechococcus sp. PCC 7942 does not prevent photoautotrophic growth, as is also observed for the equivalent mutant in Synechocystis sp. PCC 6803 (Zhang et al., 1994), but it does produce a significant decrease in the maximum capacity for photosynthetic electron transport. This drop in photosynthetic capacity is most probably linked to the slightly slower growth rates and the change in the proportion of photosystems observed under light-limiting growth conditions. It occurs despite a dramatic increase in the level of cytA expression, and presumably in Cyt c6 content, in the null mutant to compensate for the loss of plastocyanin in Synechococcus. The limitation on photosynthetic capacity is more acute after a shift to low temperature under constant light. Such a cold shift in the wild type causes inhibition of photosynthesis by slowing electron transport, and thereby decreasing the removal of electrons from PSII (Campbell et al., 1992). The intrinsically lower capacity for electron transport in ΔpetE, therefore, is further exacerbated during chilling. In certain cyanobacteria and algae, Cyt c6 has been proposed to shuttle electrons from the Cyt b6 complex to Cyt c oxidase in addition to PSI, thereby providing an alternative sink for excess electrons (Binder et al., 1984; Scherer et al., 1988). This does not, however, appear to be the case in Synechococcus sp. PCC 7942 (Peschek and Schmetterer, 1982), a conclusion supported by this study. Despite the substantially higher levels of cytA mRNA, the ΔpetE strain exhibits no significant increase in dark-respiratory rates, either under standard growth conditions or during chilling stress.

In a study based on the erroneous assumption that plastocyanin was absent from Synechococcus sp. PCC 7942, this strain has been used to successfully overexpress heterologous plastocyanin from the filamentous cyanobacterium Anabaena sp. PCC 7937 (Van der Plas et al., 1989; Geerts et al., 1994). The Anabaena petE gene, coding for a basic form of plastocyanin, was cloned into Synechococcus sp. PCC 7942 under the control of the trp promoter, thereby conferring Cu-insensitive, constitutive expression. Despite this, the stability of the newly synthesized plastocyanin was dependent on Cu (Geerts et al., 1994) in a manner similar to that described for Chlamydomonas plastocyanin (Merchant and Bogorad, 1986b; Li and Merchant, 1992). The expressed Anabaena protein enhanced electron transfer rates to PSI in both isolated thylakoids and in whole Synechococcus cells, suggesting a degree of complementation between the acidic and basic forms of plastocyanin in cyanobacteria. It has been proposed that negatively charged domains within acidic plastocyanin facilitate electron transfer to PSI via binding to the positively charged Psal subunit (Widger 1991), and that basic plastocyanins may bypass this interaction to enable fast rates of electron transfer (Geerts et al., 1994).

Given that increased levels of heterologous plastocyanin can enhance photosynthetic electron transport in Synechococcus sp. PCC 7942, and that inactivation of petE expression lowers the capacity for such activity, it is likely that plastocyanin functions as the preferred electron donor to PSI in this cyanobacterium. This would explain the relatively high level of petE expression and the stability of the petE message during normal growth compared with that of cytA (Laudenbach et al., 1990; this study). It is also consistent with the inability of increased expression of cytA to fully compensate for the absence of plastocyanin in ΔpetE, resulting in somewhat slower growth rates and significantly increased susceptibility to chilling-induced photoinhibition. Cyt c6 probably functions to complement the activity of plastocyanin in Synechococcus irrespective of gross Cu concentration rather than to completely replace it, as in other photobionts. It may also help to maintain photosynthetic electron transport, which is essential to obligate photoautotrophs such as Synechococcus during severe Cu deprivation or other types of stress. In another cyanobacterium, Synechocystis sp. PCC 6803, additional unknown carriers have been proposed that can substitute or complement the preferred electron carriers such as plastocyanin when required (Zhang et al., 1994). Indeed, such additional electron carriers occur in photosynthetic bacteria such as Rhodobacter (Fitch et al., 1989; Jenney and Dandal, 1993), and it is possible that equivalent alternative redox proteins may also exist in Synechococcus sp. PCC 7942. Conversely, an intriguing alternative may be that PSI in Synechococcus can to some extent directly reduce NADP, and therefore maintain photoautotrophy, under conditions of restricted electron flow through PSI, as was recently demonstrated in Chlamydomonas mutants lacking PSI (Greenbaum et al., 1995). If so, this may explain the slight increase in relative PSI content in ΔpetE as being part of a compensatory response including increased cytA expression.

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LITERATURE CITED


Merchant S, Bogorad L (1986a) Regulation by copper of the expression of plastocyanin and cytochrome c553 in Chlamydomonas reinhardtii. Mol Cell Biol 6: 462–469


Nakamura M, Yamagishi M, Yoshizaki F, Sugimura Y (1992) The syntheses of plastocyanin and cytochrome c-553 are regulated by...
copper at the pre-translational level in a green alga, 

*Pediastrum boryanum.* J Biochem 111: 219–224


Sandmann G, Böger P (1980) Copper induced exchange of plastocyanin and cytochrome c553 in cultures of 

_Anabaena variabilis_ and _Plectonema boryanum._ Plant Sci 17: 417–424


