Synechocystis sp PCC 6803 Strains Lacking Photosystem I and Phycobilisome Function

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To design an in vivo system allowing detailed analysis of photosystem II (PSII) complexes without significant interference from other pigment complexes, part of the psaAB operon coding for the core proteins of photosystem I (PSI) and part of the apcE gene coding for the anchor protein linking the phycobilisome to the thylakoid membrane were deleted from the genome of the cyanobacterium Synechocystis sp strain PCC 6803. Upon transformation and segregation at low light intensity (5 \(\mu\)E m\(^{-2}\) sec\(^{-1}\)), a PSI deletion strain was obtained that is light tolerant and grows reasonably well under phototrophic conditions at 5 \(\mu\)E m\(^{-2}\) sec\(^{-1}\) (doubling time \(\sim\)28 hr). Subsequent inactivation of apcE by an erythromycin resistance marker led to reduction of the phycobilin-to-chlorophyll ratio and to a further decrease in light sensitivity. The resulting PSI-less/apcE- strain grew phototrophically at normal light intensity (50 \(\mu\)E m\(^{-2}\) sec\(^{-1}\)) with a doubling time of 18 hr. Deletion of apcE in the wild type resulted in slow photoautotrophic growth. The remaining phycobilins in apcE- strains were inactive in transferring light energy to PSII. Cells of both the PSI-less and PSI-less/apcE- strains had an approximately sixfold enrichment of PSII on a chlorophyll basis and were as active in oxygen evolution (on a per PSII basis) as the wild type at saturating light intensity. Both PSI-less strains described here are highly appropriate both for detailed PSII studies and as background strains to analyze site- and region-directed PSII mutants in vivo.

INTRODUCTION

During recent years, the cyanobacterium Synechocystis sp strain PCC 6803 has been used widely and productively in functional and structural analyses of photosystem II (PSII). A main reason for this is that Synechocystis 6803 is eminently suitable for genetic modification of PSII: this cyanobacterium is a spontaneously transformable facultative (photo)heterotroph showing homologous recombination and can survive in the absence of photosynthetic activity in the presence of glucose (reviewed by Shestakov and Reaston, 1987; Williams, 1988). A large number of directed mutations have been made in PSII of this cyanobacterium (reviewed by Nixon et al., 1992; Pakrasi and Vermaas, 1992). However, detailed functional and biochemical characterization of PSII mutants generally is complex because the PSII/PSI reaction center ratio is unfavorable in this cyanobacterium (Fujita and Murakami, 1988). Even though several useful PSII preparation procedures are available for wild-type Synechocystis 6803 (Burnap et al., 1989; Noren et al., 1991; Kirilovsky et al., 1992; Nilsson et al., 1992), preparation of oxygen-evolving PSII particles from a number of mutants has been unsuccessful, possibly due to a destabilized oxygen-evolving complex in these mutants. Apart from PSI, the presence of phycobilisome components may also complicate the interpretation of experimental data. Thus, we set out to investigate the possibility of developing strains lacking PSI and/or depleted in phycobilisome components; such strains would provide a highly suitable background into which PSII mutations can be introduced.

The core of the PSII complex consists of the PSI-A and PSI-B proteins (encoded by psaA and psaB, respectively) that together harbor the reaction center and \(\sim\)100 antenna chlorophyll molecules (Golbeck, 1992). In cyanobacteria and higher plants, the psaA and psaB genes are adjacent and are cotranscribed. In cyanobacteria, targeted deletion of PSI was found to correlate with an extreme light sensitivity of the resulting mutants; this is observed both in Anabaena variabilis ATCC 29413 (Mannan et al., 1991; Toelge et al., 1991) and Synechocystis 6803 (Smart et al., 1991; Smart and McIntosh, 1993). Thus, mutants lacking PSI were grown in darkness (as can be done for Anabaena 29413); strains such as Synechocystis 6803, which cannot be propagated in complete darkness, were propagated by light-activated heterotrophic growth (LAHG). Under LAHG conditions, cells are grown in the presence of glucose in darkness except for 5 min of light every 24 hr (Anderson and McIntosh, 1991). By selection under LAHG conditions, complete genetic segregation after directed inactivation of psaA (Smart et al., 1991) or psaB (Smart and McIntosh, 1993) was obtained in Synechocystis 6803. The resulting mutants...
were reported not to grow in light and could be propagated only under LAHG conditions. However, as will be pointed out in more detail in the Discussion section, it is relatively inconvenient and possibly physiologically artifactual to grow under LAHG conditions. Therefore, the development of light-tolerant PSI-less strains would be highly advantageous.

The elimination of phycobilisomes could also be useful to simplify PSI1 studies. The phycobilisome is a large phycobilin binding protein complex that serves as a primary light-harvesting antenna for PSI1 in cyanobacteria and red algae (Bryant, 1991). The large anchor protein LCM, encoded by the apcE gene, appears to be a central component in attachment of the phycobilisome to the thylakoid membrane and in the transfer of excitation energy from phycobilins to chlorophyll in the thylakoid (Gantt, 1988). Upon inactivation or deletion of the apcE gene in the cyanobacterium Synechococcus sp strain PCC 7002, the resulting mutant is still photoautotrophic (even though it grows slower than the wild type under photoautotrophic conditions because of a decreased PSI1 antenna size), and no intact phycobilisomes can be isolated (Bryant, 1988; Bryant et al., 1990). Thus, it could be expected that inactivation of apcE in Synechocystis 6803 would lead to a very much looser attachment of phycobilisome components to thylakoids so that the remaining phycobilisome components can be washed off easily. These properties would be highly desirable for PSI1 studies in relatively intact systems, such as thylakoids.

In this study, we show that a PSI-less strain of Synechocystis 6803 can be developed by segregation in dim light. Upon deletion of apcE, this strain can grow under the same conditions as the wild type and with a similar doubling time. These strains are highly appropriate backgrounds into which PSI1 mutations can be introduced.

RESULTS

Molecular Cloning and Construction of apcE- and PSI-less Strains

To generate an apcE- strain, at least part of apcE needed to be cloned from Synechocystis 6803 so that a plasmid could be constructed carrying an antibiotic resistance marker flanked by Synechocystis 6803 apcE regions. For this purpose, a 2.0-kb apcE gene fragment from Synechocystis 6803 was amplified using the polymerase chain reaction (PCR) with two primers designed to recognize conserved regions of apcE. These conserved regions were identified by comparison of apcE sequences from Synechococcus sp strain PCC 6301 (Capuano et al., 1991), Synechococcus sp strain PCC 7002 (Bryant, 1991), Calothrix sp strain PCC 7601 (Houmard et al., 1990), and Cyanophora paradoxa (Bryant, 1988). The primers used were 5'-TATGCTATCGTAGCTGGGGATCCCAACATC-3' and 5'-CGTTCATAAGGTACCGTATCTTCCACAA-3' and were predicted to result in a 2.0-kb apcE fragment. The PCR-amplified Synechocystis 6803 DNA was cloned in pUC118 and sequenced to verify that it was highly homologous to apcE sequences from other organisms. The PCR-generated Synechocystis 6803 DNA sequence was 64 to 69% identical with apcE sequences from other cyanobacteria, but was much less homologous to any other gene in the database. Thus, we have assigned this sequence to be part of the Synechocystis 6803 apcE sequence.

A 940-bp Smal-Smal fragment within this Synechocystis 6803 apcE gene was deleted and replaced by an erythromycin resistance cassette (Elhai and Wolk, 1988). The plasmid construct (pEE25) is shown in Figure 1. This plasmid was used to transform wild-type Synechocystis 6803. Transformants were selected by screening for erythromycin resistance and were subcultured to allow segregation to occur (a single Synechocystis 6803 cell contains multiple genome copies) and thus to obtain a homogeneous genotype. As shown in Figure 2, the strain is homozygous at apcE locus and is designated as apcE-.

PCR-mediated amplification of part of the psaAB operon, which is followed by creation of a plasmid that can be used to replace part of the psaAB operon by a chloramphenicol resistance marker in Synechocystis 6803, has been described by Boussiba and Vermaas (1992). The resulting plasmid carrying a deletion in the psaAB operon was used to transform...
As shown in Figure 3, this transformant strain lacks an intact psaAB operon and is homozygous at the psaAB locus. The absence of PSI in this strain was confirmed by protein gel blotting using antibodies raised against PSI-A and PSI-B from spinach (data not shown) and by fluorescence emission measurements at 77 K (as discussed later).

To combine in one strain the lack of PSI with the deletion of apcE, the PSI-less strain was used as the host for transformation with the apcE deletion plasmid construct pEE25.

Figure 2. DNA Gel Blot Analysis of the Wild Type and apcE− Strains.
(A) Restriction maps of the apcE gene fragment from Synechocystis 6803 in the wild type and in apcE− strains. Em1, erythromycin resistance.
(B) DNA gel blot of wild type, apcE−, and PSI-less/apcE− probed with a 32P-labeled intragenic 1.9-kb Clai-Kpnl apcE fragment. DNA from the different strains was cut with HindIII (lanes 1), Clai (lanes 2), and EcoRV and Hpal (lanes 3).

Figure 3. DNA Gel Blot Analysis of the Wild Type and PSI-less Strains.
(A) Restriction maps of the psaAB operon from Synechocystis 6803 in the wild type and PSI-less strains. Cm1, chloramphenicol resistance.
(B) DNA gel blot of the wild type, PSI-less, and PSI-less/apcE− probed with a 32P-labeled intragenic 2.3-kb Ncol-HindIII fragment. DNA from the different strains was cut with Dral (lanes 1), Kpnl (lanes 2), and Ncol (lanes 3).
Erythromycin-resistant colonies were selected, and segregation was allowed to occur while the transformants were propagated at low light intensity (5 μE m⁻² sec⁻¹). As shown in Figures 2A and 3B, results of DNA gel blotting indicated that the appropriate deletions and insertions indeed had occurred in the genome of the PSI-less/apcE⁻ strain and that the appropriate wild-type genes no longer could be detected.

Also, after amplification of the EcoRV-Kpnl fragment of apcE in the apcE⁻ and PSI-less/apcE⁻ strains, and of the Ndel-Sphl fragment of psaAB in the PSI-less and PSI-less/apcE⁻ strains by PCR, the size of the fragments obtained was as expected from the size of the deletion constructs, and no trace of the wild-type fragment sizes could be observed (data not shown). These results indicated that these strains are homozygous, that only one copy per genome exists both for the psaAB operon and for the apcE gene in the wild type, and that this psaAB and/or apcE copy has been inactivated (by partial deletion) in the corresponding strains.

Reason for Light Tolerance in the PSI-less Strain

The fact that we have been able to generate fully segregated PSI-less strains under dim light conditions seems at variance with the observation that PSI-less cells grown under LAHG conditions are extremely light sensitive (Smart et al., 1991; Smart and McIntosh, 1993). There are two possible explanations for this apparent discrepancy: (1) a secondary mutation has occurred, making the PSI-less strain light tolerant; or (2) a transition of PSI-less cells from LAHG to light conditions (and vice versa) may require a lengthy adaptation period during which cell growth is inhibited. To distinguish between these possibilities, a liquid culture of the light-tolerant PSI-less strain, resulting from segregation under dim light, was transferred to LAHG conditions and propagated under these conditions for ~4 weeks (four subcultures). Then, both this strain and a PSI-less strain that always had been propagated under LAHG conditions and had never been in continuous dim light were plated out and exposed to continuous dim light (5 μE m⁻² sec⁻¹). In both cases, a lag of ~25 days was observed, after which some colonies started to grow. Eventually, the number of surviving colonies was very similar for both types of PSI-less cells. This implies that the reason PSI-less Synechocystis 6803 is light sensitive when grown under LAHG conditions is because it needs to undergo a lengthy adaptation before it can propagate under dim light conditions. There is no evidence for the occurrence of a secondary (frequent) mutation that makes our PSI-less Synechocystis 6803 strain light tolerant.

Phenotype

The apcE⁻, PSI-less, and PSI-less/apcE⁻ strains are easily distinguished from each other and from the wild type by the color of the strains. As will be discussed later, the lack of apcE in this organism leads to a loss of most phycobilins from the cell and thus to a yellow-green color of the culture. Absence of PSI-A and PSI-B leads to a loss of the PSI core complex and thus of most of the chlorophyll in the cell, while the level of phycobilisomes is not much affected. The increase in the phycobilisome-to-chlorophyll ratio results in a turquoise blue color of the PSI-less culture. The PSI-less/apcE⁻ strain displays a light yellow-green color because of the loss of most phycobilins and the PSI core complex.

Absorption Spectra

For a more quantitative analysis of the spectral features of these strains, absorption spectra of intact cells were measured. The results are presented in Figure 4. The apcE⁻ strain showed a large reduction in the 620-nm absorption maximum (originating from phycobilins) as compared to the wild type. The PSI-less strain was decreased in its 680- and 440-nm absorption peaks, reflecting a depletion in chlorophyll a due to the loss of the PSI core proteins. The phycobilin peak was virtually unaffected. The PSI-less/apcE⁻ strain showed reduction in both the phycobilin and chlorophyll absorption peaks. The relative amount of chlorophyll remaining in the PSI-less strains was difficult to estimate precisely because of the underlying phycobilin absorption, but seemed at least fourfold lower than the amount present in the wild type. In strains lacking apcE, the amount of phycobilins (as approximated by 620 nm absorption) was decreased significantly (by a factor of ~2). These results imply that absence of apcE (and thus impairment of phycobilisome assembly and attachment) by itself does not
Figure 5. Growth Curves of Wild Type and Mutant Synechocystis 6803 Strains.

Time-dependent growth of the wild type (○), the apcE- strain (▲), the PSI-less strain (●), and the PSI-less/apcE- strain (x) propagated under the following conditions.
(A) Photoautotrophic conditions at 50 μE m⁻² sec⁻¹.
(B) Photoautotrophic conditions at 5 μE m⁻² sec⁻¹.
(C) Photomixotrophic conditions at 50 μE m⁻² sec⁻¹.
(D) Photomixotrophic conditions at 5 μE m⁻² sec⁻¹.

Growth Rates

In Figure 5, the growth of the wild type and mutant strains under photoautotrophic and photomixotrophic growth conditions is presented. As shown in Figure 5A, the apcE- strain was photoautotrophic, but grew slowly (doubling time of 38 hr) in the absence of glucose at 50 μE m⁻² sec⁻¹. Photoautotrophic growth of this mutant was even slower (doubling time 5 to 6 days) at lower light intensity (Figure 5B), which is correlative evidence for a smaller PSII antenna size. As may be expected from a strain with decreased PSII antenna size, under photomixotrophic conditions the apcE- strain grew at a rate similar to that of the wild type.

The PSI-less strain did not grow photoautotrophically and, as shown in Figure 5C, it did not even grow appreciably under photoheterotrophic conditions at higher light intensity (50 μE m⁻² sec⁻¹). However, it grew reasonably well at lower light intensity (5 μE m⁻² sec⁻¹) with a doubling time of 28 hr (Figure 5D). In contrast, a PSI-less strain resulting from segregation under LAHG conditions that has not been adapted to continuous dim light (Boussiba and Vermaas, 1992) did not grow at 5 μE m⁻² sec⁻¹ (data not shown). The light sensitivity of the PSI-less strain adapted to LAHG conditions is in agreement with data obtained by Smart et al. (1991).

After inactivation of phycobilisome function in the PSI-less
strain, the resulting PSI-less/apcE− strain could be propagated well in continuous light at 50 μE m−2 sec−1: the light intensity used for the wild type (Figure 5C). This implies that a PSI-less strain has been generated that can grow reasonably fast (with a doubling time of 18 hr) under normal light conditions. The significantly decreased light sensitivity of the PSI-less strain upon decreasing the PSII antenna size implies PSII activity is the main reason for light sensitivity in the PSI-less strain.

**PSII Quantitation**

To measure the relative amount of PSII in the wild type and the other strains, herbicide binding analysis was used to determine PSII-to-chlorophyll ratios in intact cells. Diuron is a PSII-directed herbicide with one high-affinity binding site per physiologically functional PSII unit, and from the number of diuron binding sites on a chlorophyll basis, the in vivo chlorophyll-to-PSII ratio can be calculated. As illustrated in Figure 6A, the chlorophyll-to-PSII ratio in the apcf- strain (740) was similar to that in the wild type (770). As shown in Figure 6B, the PSI-less and PSI-less/apcE− strains had a chlorophyll-to-PSII ratio of 130 and 110, respectively, and had approximately six times more PSII on a chlorophyll basis than the wild type. This suggests that in wild-type Synechocystis 6803, 80 to 85% of all chlorophyll is associated with the PSI core complex. This corresponds reasonably well to the relative decrease in chlorophyll concentration seen in PSI-less strains (see Figure 3) and to other estimates regarding the relative amounts of chlorophyll associated with PSII and PSI (Fujita and Murakami, 1988). The diuron dissociation constants were similar in the wild type and these strains, indicating that dissociation of phycobilisomes and loss of the PSI reaction centers have no effect on the conformation of the herbicide binding niche in PSII.

**Fluorescence Spectra**

A convenient way to confirm that the introduced mutations have the desired effects on pigment–protein complexes is by fluorescence emission measurements at 77 K. Spectra obtained by excitation of chlorophyll at 440 nm are presented in Figure 7A. The PSI-less and PSI-less/apcE− strains yielded peaks at 685 and 695 nm, which are characteristic for PSII, whereas the 725-nm fluorescence emission peak, originating from PSI, was absent. This confirms that chlorophyll associated with the PSI reaction center core is missing in the PSI-less and PSI-less/apcE− strains.

As presented in Figure 7B, upon 590-nm excitation (exciting phycobiliproteins), cells of the wild type showed three fluorescence emission maxima when measured in 60% glycerol, which tends to functionally uncouple phycobilisomes from thylakoid components. The peaks at 645 and 665 nm originate from phycobilisome components (phycocyanin and allophycocyanin, respectively). The 685-nm peak originates from allophycocyanin B and PSI. This 685-nm peak was absent in the apcE− and PSI-less/apcE− strains. Also, the phycocyanin (645 nm) and allophycocyanin (665 nm) emission peaks were blue shifted in the apcE− and PSI-less/apcE− strains, which may be the result of a lack of proper assembly of the remaining components of the phycobilisome complex. Interestingly, at 590-nm excitation, fluorescence emission shoulders were observed at 725 nm, even in strains lacking PSI. It is likely that the 725-nm emission shoulder in this case originated from phycobilisome.

To monitor the effect of glycerol on energy transfer from the phycobilisome to PSII, the low-temperature fluorescence emission spectra upon 590-nm excitation were also measured in 25 mM of Hepes-NaOH, pH 7. The results are shown in Figure 7C. In the apcE− strains, only a single peak (at 660 nm) was observed in the absence of glycerol. Strikingly, in contrast to the wild type, no 685-nm peak and a barely discernible 725-nm shoulder were shown for the apcE− strains. This indicates the absence of efficient energy transfer from phycobilisomes to pigments in the thylakoid membrane in the absence of apcE.
It appears that in the apcE- strains the remaining phycobilins are either free or loosely associated with the thylakoid membrane: upon isolation of thylakoids from these strains, virtually all phycobilins were separated from the thylakoid fraction upon centrifugation of broken cells (data not shown). In the wild type under the same conditions, a sizeable portion of the phycobilisomes remained in the thylakoid fraction. In the absence of PSI and glycerol, the major emission maximum was at 665 nm and may represent PSII. In contrast to the observations made in the presence of glycerol, fluorescence emission originating from phycobilisome components was low, indicating efficient energy transfer from phycobilisomes to PSII. Energy transfer between phycobilisomes and PSII in the PSI-less strain appeared to be more efficient than in the wild type at low temperature in the absence of glycerol because there was a much higher relative fluorescence emission at 665 nm in the wild type than in the PSI-less strain. This apparent difference in energy transfer efficiency may be related to a difference in energy transfer states. Dark-adapted wild-type cells are in state 2 (Fork and Satoh, 1983), with inefficient energy transfer between the phycobilisome and PSII (Mullineaux et al., 1990). In contrast, dark-adapted PSI-less cells appear to be in state 1.

Electron Transport

To determine whether the remaining phycobilins in the apcE- and PSI-less/apcE- strains still were able to harvest any light energy for PSII, oxygen evolution was measured upon excitation with light from different regions of the spectrum. As illustrated in Table 1, upon 600 nm illumination (transmission maximum at 600 nm, band width 50 nm), which preferentially excites phycobilins, little oxygen evolution could be measured in the apcE- and PSI-less/apcE- strains, in spite of the fact that a reasonable amount of phycobilins remained spectrally detectable in the apcE- strains, as indicated in Figure 4. The low rate of oxygen evolution detected can be explained by light absorption from chlorophylls because the transmission band width of the filter used is rather broad (50 nm). This indicates that light absorbed by remaining phycobilins cannot be used efficiently (if at all) to excite PSII in the apcE- strains, confirming the results obtained by 77 K fluorescence emission (Figure 7).

Upon excitation at wavelengths greater than 665 nm (mainly absorbed by chlorophyll), the oxygen evolution rates of PSI-less (compared to the wild type) and PSI-less/apcE- (compared to the apcE- strain) were five to six times higher on a per chlorophyll basis, which is compatible with the results of PSII quantitation. On a per cell basis, the apcE-, PSI-less, and PSI-less/apcE- strains showed oxygen evolution rates similar to those of the wild type (Table 1).

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Figure 7. Fluorescence Emission Spectra Detected at 77 K Using Intact Cells.

(A) Emission spectra from the wild type (——) and from the PSI-less (— — —) and the PSI-less/apcE- (· · · · · ·) strains. Excitation was at 440 nm (chlorophyll excitation). Spectra were normalized to 1.0 at 685 nm for the PSI-less and PSI-less/apcE- strains and at 725 nm for the wild type.

(B) Emission spectra from the wild type (——) and from the apcE- (— — —) and PSI-less/apcE- (· · · · · ·) strains. Excitation was at 590 nm (phycobilisome excitation), and cells were suspended in 60% glycerol in 25 mM of Hepes-NaOH, pH 7.2 min before freezing. Spectra were normalized to 1.0 at the 660- to 650-nm peak.

(C) Emission spectra from the wild type (——) and from the apcE- (——), PSI-less (— — —), and PSI-less/apcE- (· · · · · ·) strains. Excitation was at 590 nm, but in contrast to the situation in (B), no glycerol was added. Spectra were normalized to 1.0 at the 665-nm peak for the wild type, the 660-nm peak for the apcE- and PSI-less/apcE- strains, and the 685-nm peak for the PSI-less strain.
Table 1. Rates of Oxygen Evolution in Cells upon Excitation at Various Wavelengths

<table>
<thead>
<tr>
<th>Strain</th>
<th>Excitation Wavelength (nm)</th>
<th>μmol O₂ (mg Chl hr⁻¹)</th>
<th>μmol O₂ (OD₇₅₀ L hr⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>600⁺</td>
<td>380</td>
<td>1150</td>
</tr>
<tr>
<td>&gt;665⁺</td>
<td></td>
<td>330</td>
<td>950</td>
</tr>
<tr>
<td>apcE⁻</td>
<td>600</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>&gt;665</td>
<td></td>
<td>290</td>
<td>870</td>
</tr>
<tr>
<td>PSI-less</td>
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<td>1200</td>
</tr>
<tr>
<td>&gt;665</td>
<td></td>
<td>2180</td>
<td>1110</td>
</tr>
<tr>
<td>PSI-less/apcE⁻</td>
<td>600</td>
<td>190</td>
<td>150</td>
</tr>
<tr>
<td>&gt;665</td>
<td></td>
<td>1970</td>
<td>1290</td>
</tr>
</tbody>
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* Data shown are the average of four experiments and reproducible within 20% of each value reported.

Light Saturation

As noted above, the size of the PSII antenna in apcE⁻ strains appears to be decreased significantly. To quantify this, oxygen evolution was measured at different light intensities. As shown in Figure 8, the apcE⁻ and PSI-less/apcE⁻ strains needed approximately a fourfold higher light intensity to obtain a similar degree of saturation as compared to the wild type. The light saturation characteristics of the PSI-less strain resembled those of the wild type at low light intensity; however, the PSI-less strain appeared more prone to photoinhibition at higher light intensity. The results obtained with the apcE⁻ strains are compatible with the notion that the apcE⁻ and PSI-less/apcE⁻ strains have a small antenna size for PSII and that light energy absorbed by phycobilins in these strains is not transferred efficiently (if at all) to PSII.

DISCUSSION

Deletion of the PSI Reaction Center Core

The unicellular cyanobacterium Synechocystis 6803 is a highly convenient system for directed mutagenesis of the PSII complex, and a PSI-less strain would be a considerable asset for detailed analysis of PSII mutants. Previous works on targeted inactivation of the PSI reaction center in Synechocystis 6803 suggested that PSI-less mutants could be obtained only under LAHG conditions (Smart et al., 1991). However, because light influences expression of PSI genes (Mullet, 1988) and is required for photoactivation (Tamura and Cheniae, 1988), properties of PSII in cells grown under LAHG conditions are not necessarily identical to those of PSII under normal laboratory light conditions. Also, in our hands, the transformability of strains grown under LAHG conditions was poor (S. Boussiba and W. Vermaas, unpublished data), and this would pose problems for routine application of strains grown under such conditions.

Therefore, it was of interest to develop a Synechocystis 6803 strain lacking PSI but retaining the capacity to propagate in light. By carrying out transformation and subsequent segregation at low light intensity (1 μE m⁻² sec⁻¹), we have obtained a genetically homozygous strain carrying a deletion of part of the psaAB operon and growing satisfactorily at low light intensity in the presence of glucose. This PSI-less strain contained no 725-nm fluorescence emission component that is characteristic for PSI (Figure 7A), lost most of its chlorophyll (Figure 4), and did not show any PSI-mediated electron flow (data not shown). Because most, if not all, of the chlorophylls associated with the PSI core complex are associated with the gene products of psaA and psaB (Bryant, 1992) and because there is no light-harvesting chlorophyll I complex in cyanobacteria, it is likely that inactivation of the psaAB operon in Synechocystis 6803 leads to a loss of all pigments associated with PSI.

In the PSI-less as well as the PSI-less/apcE⁻ strains, diuron binding assays yielded a ratio of one diuron binding site (one PSII reaction center) per 110 to 130 chlorophylls. This ratio was two- to threefold higher than could be expected from a simple addition of the number of chlorophylls associated with CP43 and CP47 (15 to 25 each; reviewed by Vermaas and Ikeuchi, 1991) and with D1 and D2 (approximately six total; Montoya et al., 1991). It is likely that chlorophyll binding proteins other than CP43 and CP47 and the PSII and PSI reaction centers...
center proteins exist in cyanobacteria; for example, another chlorophyll binding protein can be expressed under conditions of iron depletion (Laubenbach and Straus, 1988; Riethman and Sherman, 1988a, 1988b), while a 22-kD protein resembling a light-harvesting chlorophyll II protein (Kim et al., 1992; Wedel et al., 1992) also appears to be present in *Synechocystis* 6803 (Nilsson et al., 1990).

The loss of PSI did not appear to affect PSII assembly and function: in terms of oxygen evolution, all PSII centers in the PSI-less strain were as active as in the wild type, because the ratio of the rate of oxygen evolution in the wild type and this strain (on a chlorophyll basis) was similar to the ratio of the amount of PSII on a chlorophyll basis as measured through herbicide binding. Also, in a light-sensitive PSI-less *Synechocystis* 6803 mutant (with inactivated *psaA*) PSII assemblies into functional complexes (Smart et al., 1991).

It is interesting to note that the level of phycobilisomes in the cell did not change upon deletion of PSI (Figure 4). Also, upon deletion of *apcE* the PSII-to-PSI ratio was not affected (Figure 6A). This suggests that the syntheses of these three complexes (PSII, PSI, and phycobilisomes) are independently regulated.

**Light Sensitivity of PSI-less Strains**

An important question is what may be responsible for the large difference in light sensitivity in our PSI-less strain propagated in dim-light versus in the strain grown under LAHG conditions. In the transition from LAHG to dim-light conditions, these two strains showed a similar requirement for a lengthy adaptation period. After adaptation to LAHG conditions, the relatively light-tolerant strain obtained from the segregation under dim light became light sensitive and needed ~4 weeks to recover light tolerance. This adaptation time is unusually long for an organism with a usual doubling time of approximately a day or less. It is possible that during propagation under LAHG conditions, the cells strongly express particular enzymes and accumulate specific metabolites to deal with dark growth. Transition from LAHG to continuous dim-light conditions is likely to alter metabolic pathways and may lead to particular degradation or conversion products utilizing enzymes remaining from LAHG growth and those synthesized in dim continuous light. It is possible that these incongruous enzyme complements yield components poisonous to the cells. Only after an extended period needed for degradation and dilution of the LAHG enzyme machinery may growth of the PSI-less strain in dim light become possible. A similarly long adaptation period upon transfer to LAHG conditions has been observed (Boussiba and Vermaas, 1992).

**Anchor Polypeptide LCM**

Deletion of the *apcE* gene, encoding the anchor polypeptide LCM, in the *apcE⁻* and PSI-less/*apcE⁻* strains led to a functional dissociation of phycobilisomes from PSII (a loss of light energy transfer from phycobilins to PSII chlorophylls), an easy loss of phycobilins from the thylakoid fraction, and a depletion in the level of phycobilins in *Synechocystis* 6803. This appears similar to the situation in *Synechococcus* sp PCC 7002, where no intact phycobilisomes can be isolated in an *apcE⁻* strain (Bryant et al., 1990). These results are compatible with the view of the LCM being critical not only for attachment of the phycobilisome to the thylakoid and for providing a pathway of energy transfer from phycobilin in the phycobilisome to chlorophyll in the membrane but also for stable assembly of the phycobilisome subunits.

Although deletion of *apcE* led to a decrease in the phycobilin content of the cells, a measurable amount of phycobilins remained. However, this does not impede the usefulness of the PSI-less/*apcE⁻* strain for analysis of PSII mutants by fluorescence, for example, because the phycobilins are easily and quantitatively washed off upon thylakoid isolation and are not functionally coupled to PSIII.

**Decrease of Light Sensitivity upon *apcE* Deletion**

The PSI-less/*apcE⁻* strain can be propagated under standard growth conditions (at light intensities used to propagate wild-type *Synechocystis* 6803) with a doubling time only slightly longer than that of the wild type. The fact that the PSI-less strain becomes much more light tolerant upon deletion of *apcE* implies that PSII is the main reason for the light sensitivity in PSI-less strains. It is possible that overreduction of the plastoquinone (PQ) pool (as could occur in PSI-less cells if the respiratory PQH₂-oxidizing activity is insufficient to keep up with PSII activity) directly or indirectly leads to metabolic imbalances that are detrimental to cell growth. However, the observation that growth of the PSI-less strain in the presence of 20 μM of atrazine (which blocks PSII electron transfer into the PQ pool) still was impaired (data not shown) suggests that the generation of potentially toxic substances (such as singlet oxygen, chlorophyll radicals, and/or superoxide) within PSIII may also be a contributing factor, particularly if electron flow out of the PSI complex is blocked.

In any case, now that a PSI-less cyanobacterial strain has been developed that can be grown under normal light conditions, the stage has been set for introduction of site- and region-directed mutations into the various PSII genes in this background. Detailed analysis of resulting mutants can be performed in vivo or by using thylakoids without the need to prepare PSII-enriched particles.

**METHODS**

**Culture and Growth**

*Synechocystis* sp strain PCC 6803 was cultivated in BG11 medium (Rippka et al., 1979) at 30°C. Mutant cells of this organism were grown...
in BG11 supplemented with 5 mM of glucose. The light intensity at which the wild type and the PSI-less/apcE− strain were grown was 50 μE m−2 sec−1, unless indicated otherwise. Strains lacking only photosystem I (PSI) were propagated at low light intensity (5 μE m−2 sec−1). Growth of the wild-type and mutant strains was followed under photoautotrophic and photomixotrophic (with 5 mM glucose) conditions by monitoring the optical density (cell scattering) at 730 nm using a UV-160 spectrophotometer.

**Electron Transport and Herbicide Binding Measurements**

Oxygen evolution measurements and herbicide binding experiments were performed as described by Shen et al. (1993). In oxygen evolution measurements, the electron acceptor was 0.5 mM KFe(CN)₆, while 0.1 mM of 2,5-dimethyl-p-benzoquinone was added as redox mediator between thylakoids and the nonpenetrating ferricyanide. Light was provided by a 150-W xenon arc lamp. The light was filtered through water and subsequently passed through a broad-band interference filter (λmax = 600 nm, 50-nm bandwidth) or red cut-off filters, transmitting light with a wavelength above 665 nm. For herbicide binding analysis using 14C-diuron, 50 μg of chlorophyll per mL was used for the wild type and the apcE− strain and 10 μg of chlorophyll per mL for PSI-less and PSI-less/apcE− strains.

**Fluorescence Emission Spectra**

Fluorescence emission spectra were determined using a Fluorolog 2 instrument (SPEX Industries Inc., Edison, N.J.). Cells were diluted to a concentration of 5 μg for wild type and apcE− and 2 μg for PSI-less and PSI-less/apcE− of chlorophyll per mL in 25 mM of Hepes-NaOH, pH 7, in 60% (v/v) glycerol or to a concentration of 50 μg for wild type and apcE− and 10 μg for PSI-less and PSI-less/apcE− in absence of glycerol for spectra measured at liquid nitrogen temperature. The excitation and emission slitwidths were 12 and 2.4 nm, respectively.

**DNA Isolation and DNA Gel Blotting**

DNA was prepared from Synechocystis 6803 essentially as described by Williams (1988). After restriction digestion of genomic DNA, gel blotting to GeneScreen Plus (Du Pont—New England Nuclear) membranes was performed, and blots were hybridized with a 32P-labeled nick-translated Synechocystis apcE probe (a 1.9-kb ClaI-KpnI fragment) or a Synechocystis psaAB probe (2.3-kb Ncol-HindIII fragment) and washed according to the manufacturer’s recommendations.

**Thylakoids Preparation and Protein Gel Blotting**

The procedure for the preparation of the thylakoids from the wild type and mutants was described by Shen et al. (1993). Methods used for SDS–polyacrylamide gel electrophoresis and protein gel blotting were identical to those described by Vermaas et al. (1988).

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