Characterization of a *Synechococcus* sp. Strain PCC 7002 Spontaneous Mutant Strain Defective in Accumulation of Photosystem II Core Chlorophyll-Protein Complexes

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Two photosystem II-associated chlorophyll-protein complexes of *Synechococcus* sp. strain PCC 7002 were identified. Their polypeptide compositions were similar to those of chlorophyll-containing antenna complexes of other cyanobacteria. Strain GT8B did not possess the complex responsible for 695-nm fluorescence and was unable to grow photoautotrophically; hence, this complex is necessary for photosystem II function in vivo.

The cyanobacteria are procaryotes with photosynthetic functions virtually identical to those of the higher plants. Their procaryotic nature makes them attractive systems in which to study the organization of the photosynthetic apparatus. The differences in thylakoid membrane architecture between chloroplasts and cyanobacteria involve proteins peripheral to the photosystem II (PS II) and PS I core complexes (3). The antenna complexes of the cyanobacteria include both chlorophyll-protein complexes and proteins of the phycobilisome. The major chlorophyll-protein complexes of the cyanobacterium Synechococcus sp. strain PCC 7942 (7, 12) and *Synechocystis* sp. strain PCC 6714 (4) have been identified by electrophoresis of solubilized thylakoids under mildly denaturing conditions combined with fluorescence and absorption spectroscopy. Additionally, mutant cyanobacterial strains have been useful in defining roles for various thylakoid membrane components. For example, the gene which encodes the PS II core light-harvesting complex CP-47, from *Synechocystis* sp. strain PCC 6803 has been altered by site-directed mutagenesis and has been shown to be required for PS II assembly and function (15). *Synechococcus* sp. strain PCC 7002 is a transformable, unicellular cyanobacterium capable of phototrophic growth (9). This property allows for the maintenance of mutant strains defective in PS II function. In this study, we characterize the PS II-associated chlorophyll-protein complexes of *Synechococcus* sp. strain PCC 7002 and describe the isolation and characterization of a spontaneous mutant strain of *Synechococcus* sp. strain PCC 7002 which does not accumulate a homolog to the higher-plant PS II core chlorophyll-protein complex CP-47.

*Synechococcus* sp. strain PCC 7002 was grown in liquid media and on plates using ASNIII medium as described by Rippka et al. (13). The cyanobacteria were incubated at 30°C over a bank of fluorescent lights which provided a light intensity of 100 μE/m²·s. Liquid cultures were bubbled with air. Glycerol-tolerant strains were obtained by subculturing wild-type cells in liquid media containing successively increased concentrations of glycerol. Cultures growing in the presence of 20 mM glycerol were spread onto plates of ASNIII-D/G medium which contained 5 mM glycerol and the PS II inhibitor dichlorophenyl dimethyurea (10 μM) to select for glycerol-tolerant cells capable of phototrophic growth. Eleven clones (GT1 through GT11) were isolated. Long-term plate culture under phototrophic conditions gave rise to many spontaneous mutant strains, which were easily recognized by their altered appearance (16). Colonies of mutant strain GT8B were much paler green than those of the wild-type strain or its parental glycerol-tolerant strain.

*Synechococcus* sp. strain PCC 7002 chlorophyll-protein complexes. Dodecyl-β-D-maltoside extracts were prepared from *Synechococcus* sp. strain PCC 7002 cells by the method of Pakrasi et al. (12). These preparations yielded three green bands after nondenaturing polyacrylamide gel electrophoresis (4, 12) (Fig. 1, lane 1). Transillumination of the gel with 365-nm UV light revealed differences in the fluorescence properties of the chlorophyll-protein complexes. Complexes A and B were fluorescent at room temperature, while the fluorescence of the complexes of lower electrophoretic mobility was quenched (Fig. 1, lane 4). The nonfluorescent complexes probably corresponded to PS I, since PS I from cyanobacteria and chloroplasts under these conditions contains a functional P700 trap, an efficient quencher of fluorescence (10). Fluorescent bands A and B probably represent complexes containing PS II and antenna chlorophyll-protein complexes which would lack such a quenching mechanism. Bands A and B were excised and subjected to denaturing electrophoresis to examine their polypeptide composition (Fig. 1, lanes 2 and 3). Chlorophyll-protein complexes in band A contained apoproteins of 43 of 47 kilodaltons. A 36-kilodalton protein was the major species present in complex B. Similar complexes have been described previously in other species of cyanobacteria. The polypeptide composition of complex A is like that of complex IIIa of *Synechocystis* sp strain. PCC 6714 (4, 5) and complexes CPVI-2 and CPVI-3 of *Synechococcus* sp. strain PCC 7942 (12). These complexes contain the major chlorophyll-binding proteins of the PS II core, which are homologs to the chloroplast proteins CP47 and CP43 (4, 12). Complex B probably corresponds to complex IIIb of *Synechocystis* sp. strain PCC 6714 (4) and the CPVI-4 complex of *Synechococcus* sp. strain PCC 7942 (12). These complexes contain accessory chlorophyll-protein complexes which are probably unique to cyanobacteria. The CPVI-4 complex becomes the major chlorophyll-binding species when *Synechococcus* sp. strain PCC 7942 cells are grown under iron-limited conditions (12). Spontaneous mutant strain GT8B. The absorption spectra

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of wild-type and mutant strain GT8B cells are shown in Fig. 2A. Cells of the mutant strain demonstrated decreased $A_{681}$ by chlorophyll relative to the $A_{635}$ of phycocyanin. This change in pigment complement was quantitated spectrophotometrically after pigment extraction by use of known extinction coefficients for chlorophyll (1) and phycocyanin (2). A summary of these data, normalized to dry weight, is presented in Table 1. Cells of phototrophically grown glycerol-tolerant strain GT1 possessed chlorophyll levels nearly one-third greater than levels in wild-type cells grown photoautotrophically. This was a consequence of growth in the presence of dichlorophenyl dimethylurea (8). However, phototrophically grown GT8B cells possessed chlorophyll levels one-third less than levels in photoautotrophically grown wild-type cells and thus one-half those of photoheterotrophically grown cells of strain GT1. Cellular phycocyanin levels were unaffected by mode of growth or by the mutation responsible for the phenotype of strain GT8B. The phototrophic growth rate of strain GT8B, as measured by increased $A_{720}$, was unaffected by this decrease in chlorophyll content.

**Chlorophyll-protein complexes of strain GT8B.** Thylakoid membrane preparations were subjected to electrophoresis under nondenaturing conditions and photographed with 365-nm transillumination (Fig. 1, lane 5). The chlorophyll-protein complexes responsible for band A were not present in the mutant strain, while band B was readily visualized. The electrophoretic pattern obtained from Coomassie-stained, denaturing polyacrylamide gels of thylakoid preparations, however, revealed no obvious differences between the wild-type and the mutant strains (data not shown). Mutant strain GT8B was incapable of photoautotrophic growth, and revertant colonies were never obtained. This suggests that the chlorophyll-protein complexes present in band A are necessary for PS II function or assembly.

**Spectroscopic studies.** Fluorescence emission spectra were measured.
obtained with an SLM 8000 spectrofluorimeter (SLM Corp., Urbana, Ill.). The low-temperature (77 K) fluorescence emission spectra of GT1 and GT8B cells grown phototrophically are compared in Fig. 2B. Excitation was at 435 nm, a wavelength preferentially absorbed by chlorophyll. The trace for GT1 cells shows characteristic PS II-associated emission peaks at 685 and 695 nm. The relative fluorescence at these wavelengths was large with respect to the PS I-associated fluorescence at 719 nm. This type of chlorophyll organization, resulting in increased PS II antenna size, is a consequence of forced phototrophic growth by inclusion of the PS II inhibitor dichlorophenyl dimethylurea in the culture medium (8). Strain GT8B cells possess the chlorophyll-protein complex responsible for the 685-nm fluorescence but clearly lack the 695-nm fluorescing species. Figure 2C shows the fluorescence emission spectra obtained by excitation at 620 nm, a wavelength preferentially absorbed by cyanobacterial phycobilisomes. Wild-type cells demonstrated greater fluorescence from uncoupled phycobilisomes at 665 nm than at 686 nm. These cells also demonstrated fluorescence at 696 nm. Strain GT8B yielded greater relative fluorescence at 686 nm than at 665 nm. Again, the 696-nm fluorescence peak attributed to chlorophyll molecules closely associated with the PS II core was missing from the spectrum of mutant strain GT8B. Since Nakatani et al. (11) and Pakrasi et al. (12) have demonstrated in vitro that the 696-nm fluorescence arises from CP-47 and its homologs, strain GT8B is probably defective in the assembly of CP-47 to the PS II core. Additionally, spillover of excitation energy to PS I, monitored by the decreased 717-nm fluorescence of GT8B cells relative to that of wild-type cells, appears to be diminished (Fig. 2C).

These data strongly implicate the chlorophyll-protein complexes of band A, containing the homolog to CP-47 of Synechococcus sp. strain PCC 6803, as the origin of the in vivo 696-nm fluorescence signal. The results also suggest a close physical association between phycobilisomes and CP-47, since there was minimal spillover of 620-nm excitation energy to PS I in cells of strain GT8B.

Long-term phototrophic growth has been shown to give rise to a wide spectrum of mutant cyanobacterial strains with altered phycobilisomes and chlorophyll complements (16). These strains could prove useful in examining the assembly and biophysical associations of thylakoid membrane components. Recent work by Vermaas et al. (14) suggests a central role for CP-47 in the assembly of the PS II core complex. The measurement of primary-quinone reduction (6) could determine whether the PS II reaction centers of strain GT8B assemble in the absence of CP-47.

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LITERATURE CITED


TABLE 1. Absorption spectra of wild-type and mutant strain GT8B cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium</th>
<th>Pigment/cell ratio (wt/wt)* ± SE (% control)</th>
<th>PC Chl</th>
<th>PC/Chl ratio (wt/wt)</th>
<th>Growth rate (generations/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT7002</td>
<td>ASIII</td>
<td>2.2 ± 0.0 (100)</td>
<td>7.6 ± 0.10 (100)</td>
<td>28</td>
<td>2.08</td>
</tr>
<tr>
<td>GT1</td>
<td>ASIII-D/G</td>
<td>2.3 ± 0.20 (105)</td>
<td>9.8 ± 0.05 (129)</td>
<td>24</td>
<td>1.64</td>
</tr>
<tr>
<td>GT8B</td>
<td>ASIII-D/G</td>
<td>2.3 ± 0.10 (105)</td>
<td>5.0 ± 0.10 (66)</td>
<td>46</td>
<td>1.69</td>
</tr>
</tbody>
</table>

* Grams of phycocyanin (PC) and chlorophyll (Chl) per gram (dry weight) of cells (10^2 and 10^4, respectively).