The BtpA Protein Stabilizes the Reaction Center Proteins of Photosystem I in the Cyanobacterium *Synechocystis* sp. PCC 6803 at Low Temperature[1]

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Specific inhibition of photosystem I (PSI) was observed under low-temperature conditions in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Growth at 20°C caused inhibition of PSI activity and increased degradation of the PSI reaction center proteins PsaA and PsaB, while no significant changes were found in the level and activity of photosystem II (PSII). BtpA, a recently identified extrinsic thylakoid membrane protein, was found to be a necessary regulatory factor for stabilization of the PsaA and PsaB proteins under such low-temperature conditions. At normal growth temperature (30°C), the BtpA protein was present in the cell, and its genetic deletion caused an increase in the degradation of the PSI reaction center proteins. However, growth of *Synechocystis* cells at 20°C or shifting of cultures grown at 30°C to 20°C led to a rapid accumulation of the BtpA protein, presumably to stabilize the PSI complex, by lowering the rates of degradation of the PsaA and PsaB proteins. A *btpA* deletion mutant strain could not grow photoautotrophically at low temperature, and exhibited rapid degradation of the PSI complex after transfer of the cells from normal to low temperature.

Thylakoid membranes of cyanobacteria, algae, and plants contain four major multisubunit protein complexes: photosystem I (PSI), photosystem II (PSII), the cytochrome b_6/f complex, and ATP synthase. During oxygenic photosynthesis, the PSI complex functions at the reducing end of the photosynthetic electron transfer chain as a plastocyanin-ferredoxin oxidoreductase. Over the past decade, biochemical, genetic, and biophysical studies have led to a detailed understanding of the composition, structure, and functions of this protein complex (Chitnis, 1996). The structure of a crystallized PSI complex from *Synechococcus elongatus*, a thermophilic cyanobacterium, has been solved to a 4 Å resolution (Krauss et al., 1996). The structure and function of various protein subunits of PSI have also been examined in numerous genetic studies using site-directed mutants of cyanobacteria and the green alga *Chlamydomonas reinhardtii* (Pakrasi, 1995; Chitnis, 1996), as well as in studies utilizing biochemical approaches such as protease treatment (Sun et al., 1997), epitope mapping (Xu et al., 1994), and cross-linking experiments (Muhlenhoff et al., 1996).

Significantly less is known about the process of assembly of PSI (Wollman et al., 1999) and the effects of different environmental conditions such as light and temperature on the form and function of this protein complex. It has been accepted for a long time that the principal site of photoinhibition in chloroplasts of higher plants and algae and in cyanobacteria is localized in PSII (Powles, 1984). However, a number of studies have indicated that in green plants, PSI is an important target of photoinhibitory damage in vivo under relatively weak light and chilling temperature, conditions under which PSII is not appreciably affected (Terashima et al., 1994; Sonoike et al., 1995; Tjus et al., 1998). Selective photoinhibition of PSI has also been demonstrated in thylakoid membranes isolated from both chilling-sensitive and chilling-tolerant plants (Sonoike, 1995). However, little is known about photoinhibition of PSI at low temperature in cyanobacterial cells. The underlying molecular mechanism of inhibition of PSII under high light and low temperature is an enhanced rate of damage of the reaction center protein D1 (Aro et al., 1993). The mechanism of PSI inhibition under relatively weak light conditions and chilling temperature is not well understood, although some models explaining this phenomenon have been proposed (for review, see Sonoike, 1998). The major events during photoinhibition of PSI seem to be the same as for photoinhibition of PSII: inhibition of the electron acceptor side, inactivation of the reaction center, then degradation of the reaction center subunit proteins (for review, see Sonoike, 1996b).

The cyanobacterium *Synechocystis* sp. strain PCC 6803 offers an excellent model system with which to study the biogenesis, form, and function of both PSI and PSII protein complexes (Pakrasi, 1995). To date, it is the only photosynthetic organism whose genome sequence has been completely determined. Recently, we have described BtpA, a PSI-complex-specific regulatory protein in *Synechocystis* 6803 (Bartsevich and Pakrasi, 1997). The *btpA* gene was identified during

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genetic complementation of a random photosynthesis-deficient mutant strain (Bartsevich and Pakrasi, 1995). This mutant, BP26, has a reduced PSI content but a normal level of PSII. The genetic lesion in the BP26 mutant is a missense mutation resulting in the replacement of a Val residue by a Gly residue in the BtpA protein (Bartsevich and Pakrasi, 1997). We have also demonstrated that BtpA is an extrinsic thylakoid membrane protein exposed to the cytoplasm of *Synechocystis* 6803 cells (Zak et al., 1999).

In the present study we describe, for the first time to our knowledge, the damaging effect of low temperature on PSI in the cyanobacterium *Synechocystis* 6803. In addition, we demonstrate that the BtpA protein is required for the enhanced stability of PsaA, a reaction center protein of PSI (and consequently the entire PSI complex) at low temperature.

RESULTS

Influence of Low Temperature on Growth Characteristics of Wild-Type and BP26 Mutant Cells

The BP26 mutant was isolated using a Glc-sensitive wild-type strain of *Synechocystis* 6803 (Bartsevich and Pakrasi, 1997). Under the photoautotrophic conditions used in this study, this wild-type strain had an approximately 12-h doubling time at 30°C (Table I). When the growth temperature was lowered to 20°C, these cells grew 2.3 times slower. In comparison, at 30°C, the BP26 mutant cells grew four times slower than their parental wild-type cells. Interestingly, at 20°C, the growth rate of the mutant strain was nearly the same as that of the wild-type strain. The *btpA*-deletion (Δ*btpA*) mutant grew at the same rate as that of the BP26 mutant at 30°C. However, the growth of the deletion mutant under photoautotrophic conditions was completely inhibited at 20°C.

Spectroscopic and Polarograhic Analysis of Wild-Type and *btpA* Mutant Strains Grown at Normal and Low Temperatures

Similar to our previous observations (Bartsevich and Pakrasi, 1997), at 30°C, the BP26 mutant cells had a significantly reduced chlorophyll content compared with the wild-type cells (Table I). Since the majority of chlorophyll molecules in cyanobacterial cells are bound to PSI (Nyhus et al., 1993), these data suggested a reduced level of PSI in the mutant strain. Interestingly, growth at 20°C resulted in a nearly 2-fold decrease in the chlorophyll content of the wild-type cells, whereas that of the BP26 mutant cells increased 2-fold. On a chlorophyll basis, this mutant strain grown at 30°C had a significantly reduced content of P700, the reaction center molecules of PSI. However, growth at 20°C led to a significant decrease in the P700 content of the wild-type cells, whereas that of the BP26 mutant increased.

Using artificial electron donors and acceptors, PSII- and PSI-mediated electron transport rates were examined in intact cells of wild-type and mutant strains. As shown in Table I, the PSII-mediated oxygen evolution rates were similar in wild-type and mutant cells grown at 30°C and 20°C. In contrast, the PSI activity was almost two times lower in wild-type cells grown at 20°C compared with those grown at 30°C. As expected, compared with wild-type cells, the BP26 cells grown at 30°C had a 4-fold-reduced PSI activity. However, at 20°C, this strain had a nearly normal PSI activity. The spectroscopic properties and electron transport activities of the Δ*btpA* mutant cells grown at 30°C were nearly identical to those of the BP26 mutant cells grown at the same temperature.

We used low-temperature fluorescence emission spectroscopy to determine the relative contents of PSI and PSII complexes in the wild-type and mutant cells. As shown in Figure 1, there was a decrease in the level of fluorescence at 726 nm from wild-type cells grown at 20°C compared with those at 30°C, indicating that the content of PSI centers was lower at 20°C. In contrast, lowering of growth temperature resulted in a significant increase in the fluorescence yield at 726 nm from the BP26 mutant cells, an observation that was consistent with the electron transport data mentioned above. Measurements of variable fluorescence induction yield showed that there was no significant difference in the relative amounts

Table I. *Influence of growth temperature on doubling time, chlorophyll content, electron transport activities, and contents of PSI and PSII in wild-type (WT), BP26, and Δbtp A mutant cells of Synechocystis 6803*

Each value is the mean ± SD of at least three independent measurements. Asc, Sodium ascorbate; DAD, 3,6-diaminodurene; DCBQ, 2,6-dichloro-*p*-bezoquinone; FeCN, potassium ferricyanide; F_v, variable fluorescence; F_m, maximum fluorescence; MV, methyl viologen.

	WT		BP26		Δ*btpA* 30°C
	30°C	20°C	30°C	20°C	
Doubling time in BG11 (h)	11.8 ± 0.3	27.3 ± 0.6	49.8 ± 1.3	24.7 ± 0.6	53.0 ± 1.4
Chlorophyll (μg/10^8 cells)	34.5 ± 1.3	17.3 ± 0.9	8.3 ± 0.5	12.9 ± 1.1	9.0 ± 0.2
Chl/P700 (mol/mol)	184.7 ± 4.0	271 ± 3.6	579.7 ± 9.0	388.3 ± 3.1	575 ± 2.8
Electron transport rates (μmol O_2 10^{-8} cells h^{-1})					
H_2O to DCBQ/FeCN (PSII)	12.3 ± 0.4	11.3 ± 0.15	11.5 ± 0.25	12.3 ± 0.15	11.1 ± 0.2
DAD/Asc to MV (PSI)	8.9 ± 0.15	4.3 ± 0.15	2.2 ± 0.2	3.8 ± 0.1	2.2 ± 0.07
Chlorophyll fluorescence induction					
F_v/F_m (relative units)	0.37 ± 0.04	0.36 ± 0.03	0.31 ± 0.03	0.30 ± 0.03	0.35 ± 0.01

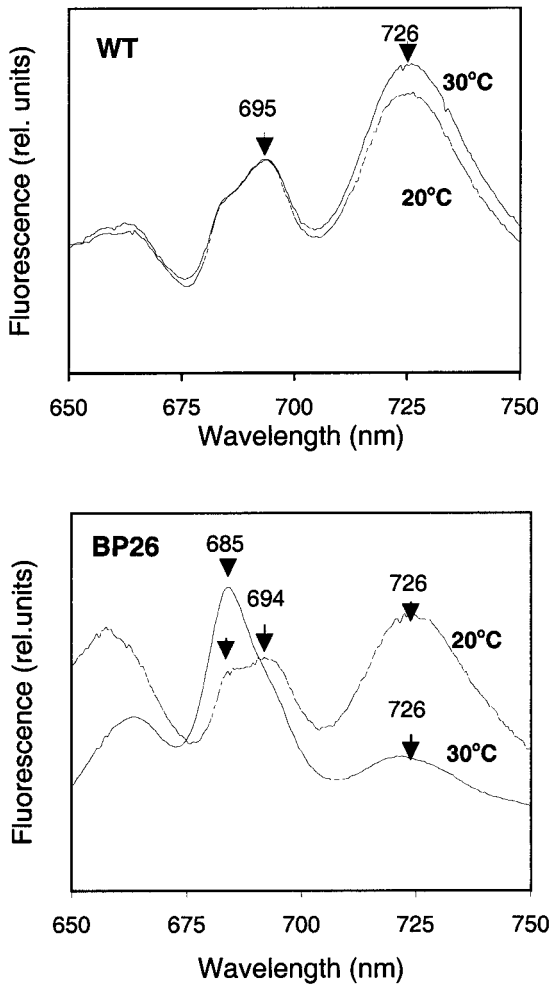

Figure 1. Fluorescence emission spectra (77 K) from whole cells of wild-type (WT) and BP26 mutant strains grown at 30°C or 20°C. Excitation was at 600 ± 5 nm. The spectra were corrected for the wavelength characteristics of the emission monochromator and the response of the signal detector. See "Materials and Methods" for experimental details.

of active PSII centers in wild-type and BP26 cells grown at 30°C or 20°C (Table I).

In summary, the PSI content of the wild-type cells significantly decreased when growth temperature was lowered from 30°C to 20°C. On the other hand, the BP26 mutant had a low PSI titer at 30°C, which increased at 20°C.

Effects of Growth Temperature on Steady-State Levels of PSI and PSII Proteins

At 30°C, both the BP26 and Δ*btpA* mutants had reduced levels of a majority of the PSI protein subunits, including PsaA, PsaB, PsaC, PsaF, and PsaL (Fig. 2). In contrast, the amount of CP43, a component of PSII, remained unaltered. Interestingly, the PsaD protein of PSI was present at the same level in wild-type, BP26, and Δ*btpA* mutant strains. The PsaK protein was not detected in either of the *btpA* mutants. However, we do not think that the absence of this protein is the primary reason for impaired PSI activity in these mutants, since directed inactivation of the *psaK* gene does not affect PSI function in *Synechocystis* 6803 cells (Nakamoto and Hasegawa, 1999). It is noteworthy that the Δ*psaAB* deletion mutant strain had a protein profile distinctly different from that of the BP26 mutant. For example, the PsaD protein is absent in the Δ*psaAB* mutant, whereas the PsaF and PsaL proteins are present in normal amounts. In the missense mutant BP26, the amount of the BtpA

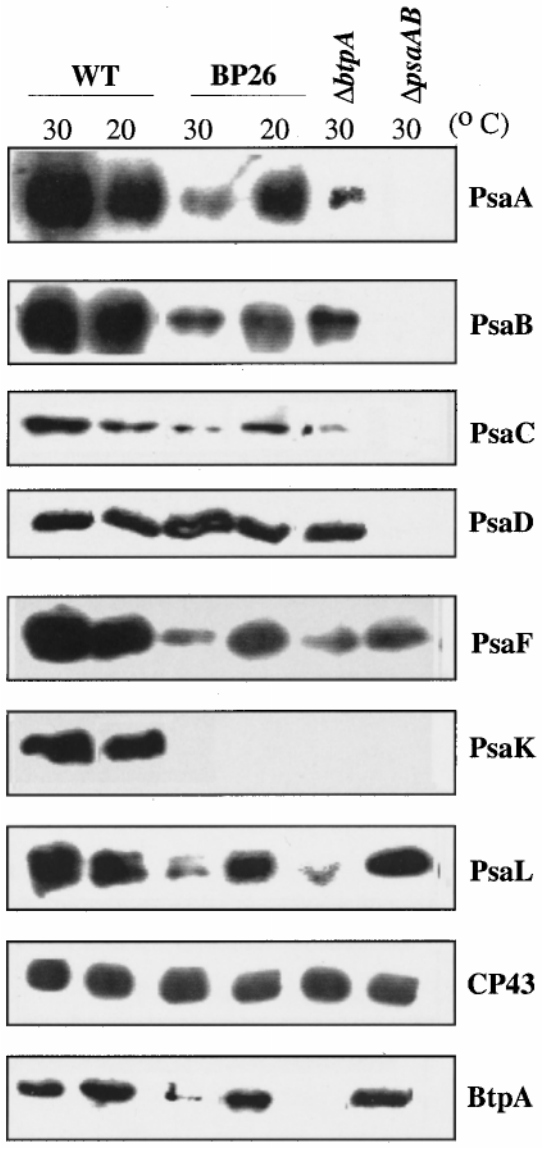

Figure 2. Presence of PSI and PSII proteins in wild-type and mutant strains grown at 30°C or 20°C. Membrane proteins from wild-type (WT), BP26, btpA-deletion (Δ*btpA*), and *psaA/B*-deletion (Δ*psaAB*) strains were fractionated on SDS-PAGE (50 μg protein per lane), transferred to nitrocellulose filters, and immunostained with antibodies against PsaA, PsaB, PsaC, PsaD, PsaF, PsaK, PsaL, CP43, and BtpA proteins, respectively.

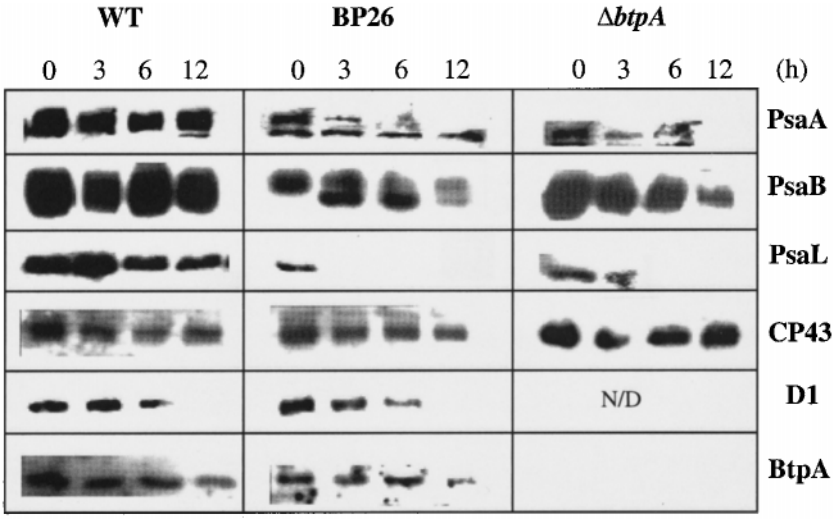

Figure 3. Stability of PSI and PSII proteins at 30°C. Wild-type (WT), BP26, and *btpA*-deletion (Δ*btpA*) strains were incubated in the presence of chloramphenicol (150 μg/mL), and aliquots of cells were collected immediately (0), and 3, 6, and 12 h later. Membrane proteins were fractionated on SDS-PAGE, transferred to nitrocellulose filters, and immunostained with antibodies against PsaA, PsaB, PsaL, CP43, D1, and BtpA proteins, respectively. Seventy micrograms of protein-containing sample was loaded in each lane. N/D, Not determined.

protein was approximately 40% of that in the wild-type cells. However, it was present in normal amount in the Δ*psaAB* strain.

Lowering the growth temperature to 20°C caused significant changes in the protein contents of both wild-type and BP26 mutant cells. In both strains, we observed significant increases in the amount of the BtpA protein. In the BP26 mutant, the levels of PsaA, PsaB, PsaC, PsaF, and PsaL proteins were increased, while the amount of CP43 protein was not changed. As mentioned before, the Δ*btpA* mutant did not grow autotrophically at 20°C. Therefore, it was not possible to examine the effects of such low-temperature growth on its protein profile. We also examined the steady-state levels of mRNAs for PSI proteins at both temperatures. Our data indicated that neither lowering the growth temperature from 30°C to 20°C nor mutations in the BP26 and Δ*btpA* strains affected the transcript levels of the *psaAB*, *psaK*, and *psaL* genes (data not shown).

Rates of Degradation of PSI and PSII Proteins at Different Temperatures in Wild-Type, BP26, and *btpA* Mutant Strains

To examine the rates of degradation of various proteins of PSI and PSII at different temperatures and the possible role of the BtpA protein in this process in *Synechocystis* 6803, we added chloramphenicol to these cyanobacterial cells to inhibit protein synthesis. As shown in Figure 3, the rates of degradation of various PSI proteins were significantly higher in the BP26 and Δ*btpA* mutant cells than in the wild-type cells at 30°C. In both of these mutants, the majority of the PsaA protein was degraded within 3 h of incubation with chloramphenicol, while most of the PsaB protein was degraded in 12 h. In the BP26 mutant, the PsaL protein could not be detected after 3 h. In contrast, the PsaA, PsaB, and PsaL proteins were stable in the wild-type cells for at least 12 h. The BtpA protein was almost equally stable in the wild-type and BP26 strains, although it was present in a significantly lower amount in the mutant cells. Moreover, the rates of degradation of CP43 and D1, two PSII proteins, were similar in all three strains grown at 30°C.

Inhibition of protein synthesis in BP26 and wild-type cells grown at 20°C showed that various PSI proteins have similar half-lives in both of these strains (Fig. 4). Therefore, in the BP26 mutant, the PsaA, PsaB, and PsaL proteins were more stable at 20°C than at 30°C.

In the experiments described above, we determined the rates of degradation of various proteins in cultures that were constantly grown at 30°C or 20°C. Next, we grew cells at 30°C, added chloramphenicol, and transferred the cultures to 20°C for 24 h. In the absence of chloramphenicol, the BtpA protein accu-

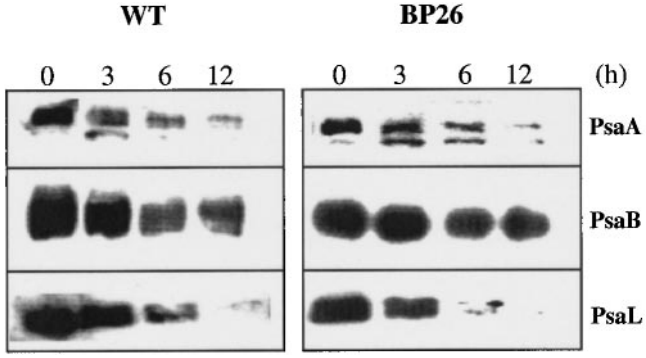

Figure 4. Stability of PSI and PSII proteins at 20°C. Wild-type (WT) and BP26 mutant strains were incubated in the presence of chloramphenicol (150 μg/mL), and aliquots of cells were collected immediately (0), and 3, 6, and 12 h later. Membrane proteins were fractionated on SDS-PAGE, transferred to nitrocellulose filters, and immunostained with antibodies against PsaA, PsaB, and PsaL proteins, respectively. Seventy micrograms of protein-containing sample was loaded in each lane.

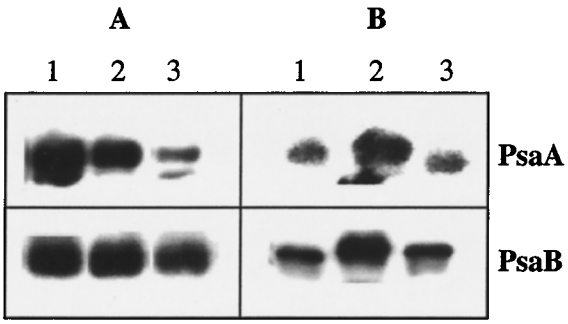

Figure 5. Changes in the amounts of PSI proteins after a temperature shift from 30°C to 20°C. Wild-type (A) and BP26 (B) mutant cells were grown at 30°C and then transferred to 20°C for 24 h without (lanes 2) or with (lanes 3) chloramphenicol (150 μg/mL). Lanes 1, Control cells maintained at 30°C. Membrane proteins were separated on SDS-PAGE, transferred to nitrocellulose membranes, and probed with antibodies against PsaA and PsaB proteins, respectively. Eighty micrograms of protein-containing sample was loaded in each lane.

mulated at 20°C in both wild-type and BP26 cells (data not shown). As a result of the same treatments, the level of the PsaA protein in the wild-type strain decreased slightly (Fig. 5), while the amounts of the PsaB (Fig. 5) and PsaL (data not shown) proteins remained practically unchanged. In contrast, in the BP26 mutant, there was a significant increase in the amounts of the PsaA and PsaB proteins. As shown in Figure 5, such increases did not take place when chloramphenicol was added before the transfer of the cultures to 20°C. Thus, new protein synthesis was necessary for the enhanced accumulation of the PSI reaction center proteins in the BP26 mutant cells.

Since the Δ*btpA* mutant could not grow autotrophically at 20°C, we transferred the culture grown at 30°C to 20°C and examined the levels of various PSI and PSII proteins after different periods of incubation at the lower temperature. The PsaA protein was rapidly degraded within the first 6 h (Fig. 6). The rate of degradation of the PsaB protein was slightly slower, although this protein was at a very low level after 12 h. During the same period of time, the levels of these two proteins in the wild-type cells did not change significantly (data not shown). Moreover, the content of the manganese-stabilizing protein (MSP) of PSII did not change during 24 h of incubation of the Δ*btpA* mutant cells at 20°C (Fig. 6). Therefore, the presence of the BtpA protein was necessary for the stability of the reaction center proteins of PSI in *Synechocystis* 6803 cells.

DISCUSSION

Our data clearly demonstrate that at low temperature, a major target for photoinhibition in the thylakoid membranes of the cyanobacterium *Synechocystis* 6803 is the PSI complex, not PSII. For any organism, chilling temperature is usually considered to be 10°C to 15°C below its normal growth temperature. The normal temperature range for the growth of *Synechocystis* 6803 is 30°C to 34°C, so 20°C may be considered a chilling temperature for this organism. The effects of chilling temperature and relatively low light on the inhibition of PSI were previously documented for cold-sensitive (Terashima et al., 1994) and recently for cold-resistant (Tjus et al., 1998) plants. Our data demonstrate, for the first time to our knowledge, that chilling temperature induces partial damage of the PSI complex in the cyanobacterium *Synechocystis* 6803. For the wild-type cells of *Synechocystis* 6803, growth at 20°C led to a decrease in chlorophyll and P700 content, and the PSI activity was inhibited almost 2-fold. However, PSII activity and the cellular content of PSII centers were the same as at 30°C. In particular, the levels of many PSI proteins were significantly reduced at 20°C, while those of PSII proteins remained essentially the same at both temperatures.

A significant conclusion of this study is that the BtpA protein is an important regulatory factor that stabilizes the reaction center core complex of PSI. The BP26 mutant, with a missense mutation in the *btpA* gene, and the Δ*btpA* mutant, from which the *btpA* gene has been deleted, have similar growth rates, electron transport activities, and chlorophyll, P700, and protein levels at 30°C. Both mutants were impaired in their PSI activity without any noticeable reduction in PSII activity. In particular, the steady-state levels of most of the PSI proteins were significantly reduced. Interestingly, there were significant differences in the profiles of PSI proteins in the PSI reaction center deletion mutant (Δ*psaAB*) strain and the *btpA* mutant strains. For example, the thylakoid membranes from the former mutant did not have any detectable level of PsaD and PsaC proteins, whereas these proteins are present in the BP26 and Δ*btpA* strains. On the other hand, the Δ*psaAB* strain accumulated a normal amount of PsaL, a protein that was present in a significantly reduced level in the *btpA*

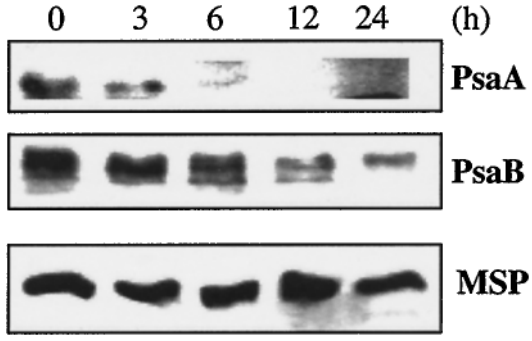

Figure 6. Changes in the amounts of PsaA, PsaB, and MSP proteins in the *btpA*-deletion (Δ*btpA*) strain during temperature shift from 30°C to 20°C. The deletion mutant was grown at 30°C and then transferred to 20°C. Aliquots of cells were collected before transfer (0), and 3, 6, 12, and 24 h after incubation at 20°C. Membrane proteins were fractionated on SDS-PAGE, transferred to nitrocellulose membranes, and probed with antibodies against PsaA, PsaB, and MSP proteins, respectively. Eighty micrograms of protein-containing sample was loaded in each lane.

mutant strains. It has been shown that deletion of the *psaL* gene has no effect on the accumulation or activity of reaction center proteins of the PSI complex (Schluchter et al., 1996), but only on the assembly of PSI trimers in cyanobacterial cells (Xu et al., 1995). Thus, the reduced PSI activity of the *btpA* mutants cannot be explained by a reduction in the level of PsaL. It is possible that the PsaL protein that is not yet assembled in the PSI complex is relatively stable (as in the Δ*psaAB* mutant). However, in the *btpA* mutants, PsaL is first assembled into the PSI complex, and is subsequently degraded as a consequence of the instability of the assembled complex. The level of the BtpA protein almost doubled in wild-type cells at 20°C (Fig. 2), suggesting its protective role for the reaction center proteins of PSI. Altogether, these data lead to the conclusion that the BtpA protein is a necessary factor for a stable maintenance of the reaction center core proteins of PSI, especially at low temperature.

The phenotypic differences between the two mutant strains BP26 and Δ*btpA* became obvious at low temperature. When grown at 20°C, the BP26 mutant grew better, had an elevated content of chlorophyll and P700, and higher amounts of various PSI proteins compared with plants grown at 30°C. It also was able to accumulate the BtpA protein at an enhanced level. In contrast, the Δ*btpA* mutant could not grow autotrophically at 20°C. The transfer of this strain from 30°C to 20°C resulted in rapid degradation of the reaction center proteins of PSI. These findings underscore the essential role of the BtpA protein in the stabilization of the PSI complex at 20°C. The BtpA protein is not present in the deletion mutant, so the PSI complex is extremely unstable at low temperature (Fig. 6). On the other hand, the Val-51 to Gly mutation in the BP26 mutant (Bartsevich and Pakrasi, 1997) presumably makes the BtpA protein temperature sensitive, so that it functions poorly at 30°C but normally at 20°C.

It is noteworthy that in the *btpA* mutants, the PsaA protein had a significantly higher degradation rate than its partner in the PSI reaction center heterodimer, PsaB. We hypothesize that the BtpA protein is primarily involved in maintaining the stability of the PsaA protein in thylakoid membranes of *Synechocystis* 6803, especially at low temperature. So far, it has been shown that in higher plants and algae, the stability of the PsaB protein is more critical than that of PsaA. For example, in *C. reinhardtii*, it has been demonstrated that in the absence of the synthesis of the PsaB protein, PsaA cannot be detected whereas in the absence of the synthesis of PsaA, one can still detect the PsaB protein in the thylakoid membrane (Stampacchia et al., 1997). Sonoike (1996a) demonstrated specific degradation of the PsaB protein during photoinhibition of PSI in spinach thylakoid membranes. In contrast, in the present study, we observed that in the absence of the *BtpA* protein and at low temperature, degradation of the PsaA protein preceded that of PsaB.

The molecular mechanism of action of the BtpA protein is unknown. The protein does not exhibit any significant sequence similarity with any other protein of known function(s). The BtpA protein may function as a chaperone, directly interacting with the PsaA and/or PsaB proteins. In particular, BtpA may be involved in the insertion/assembly of cofactors in PSI, such as iron-sulfur centers or phylloquinones. On the other hand, it may stabilize PSI reaction center proteins indirectly. For example, BtpA may activate or stabilize the functions of oxygen-scavenging enzymes whose activities are critical for the protection of the PSI complex from photoinhibition (Sonoike, 1996, 1998). The latter, more general, role of BtpA is attractive, since homologs of the BtpA protein are present in many other non-photosynthetic organisms, including archaebacteria and nematodes (Bartsevich and Pakrasi, 1997).

MATERIALS AND METHODS

Cyanobacterial Strains and Culture Conditions

The wild-type strain of *Synechocystis* sp. PCC 6803 was grown in BG11 medium (Allen, 1968). The *psaAB* deletion mutant (Δ*psaAB*) was generated by replacing the entire coding regions for the PsaA and PsaB proteins (from nucleotide positions −46 to 4,707; Smart and McIntosh, 1991) with a kanamycin resistance cassette. The medium for the BP26 mutant was supplemented with 5 mM Glc. The *btpA* deletion mutant (Δ*btpA*) was maintained on solid BG11 medium supplemented with 10 mM Glc and 50 μg/mL kanamycin (Zak et al., 1999). The Δ*psaAB* strain was grown heterotrophically under low-light conditions (2 to 3 μmol m^{-2} s^{-1}) in the presence of 5 mM Glc and 50 μg/mL kanamycin. All other strains were cultivated under 50 μmol m^{-2} s^{-1} of white fluorescent light. Growth of wild-type and mutant strains was monitored by measurement of light scattering at 730 nm on a spectrophotometer (model DW2000, SLM-Aminco Instruments, Urbana, IL).

Measurement of Rates of Electron Transfer Reactions

Rates of photosynthetic electron transfer reactions were measured on a Clark-type oxygen electrode, essentially as described previously (Mannan and Pakrasi, 1993). Samples in BG11 medium were adjusted to equal numbers of cells that corresponded to 5 μg chlorophyll/mL for wild-type cells. The concentration of chlorophyll was measured after methanolic extraction (MacKinney, 1941).

Optical Analyses

Time-based fluorescence measurements were performed on a dual-modulation kinetic fluorometer (FL-100, Photon Systems Instruments, Brno, Czech Republic) essentially as described previously (Meetam et al., 1999). Samples were adjusted to equal numbers of cells corresponding to 1 μg

chlorophyll/mL for wild-type cells. The molar concentration of P700, the reaction center chlorophylls of PSI, was quantitated from ascorbate-reduced minus ferricyanide-oxidized chemical difference spectra of thylakoid membranes, as described previously (Mannan et al., 1991). Fluorescence emission spectra at 77 K were recorded using a fluorometer (Fluoromax 2, Instruments S.A. Inc., Edison, NJ). The samples contained intact cyanobacterial cells (10–20 μg chlorophyll/mL) in BG-11 medium.

Membrane Isolation, Electrophoresis, and Immunodetection

Cellular membranes were isolated as described previously (Zak et al., 1999). Proteins were fractionated by SDS-PAGE (Laemmli, 1970). Protein concentrations were estimated using a protein determination kit (Pierce Chemical, Rockford, IL). Proteins were blotted onto nitrocellulose filters, reacted with antisera, and the signals were visualized using enhanced chemiluminescence reagents (Pierce).

Inhibition of Protein Synthesis

Wild-type, BP26, and Δ*btpA* strains were grown in BG11 medium, and chloramphenicol was added to a final concentration 150 μg/mL. Aliquots of cells were collected immediately after the addition of chloramphenicol (0 h), as well as after incubation with the inhibitor for different periods of time.

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