Thermal Protection of the Oxygen-Evolving Machinery by PsbU, an Extrinsic Protein of Photosystem II, in *Synechococcus* species PCC 7002¹

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The evolution of oxygen is the reaction that is the most susceptible to heat in photosynthesis. We showed previously that, in the cyanobacterium *Synechococcus* sp. PCC 7002, some protein factors located on the thylakoid membranes are involved in the stabilization of this reaction against heat-induced inactivation, and we identified cytochrome C₅₅₉ as one such factor (Y. Nishiyama, H. Hayashi, T. Watanabe, N. Murata [1994] Plant Physiol 105: 1313–1319). In the present study we purified another protein that appears to be essential for the stabilization of the oxygen-evolving machinery. The purified protein had an apparent molecular mass of 13 kD, and the gene encoding the 13-kD protein was cloned from *Synechococcus* sp. PCC 7002 and sequenced. The deduced amino acid sequence revealed that the protein obtained was homologous to PsbU, an extrinsic protein of the photosystem II complex, which has been found in thermophilic species of cyanobacteria. Western analysis showed that the level of PsbU in thylakoid membranes was constant, regardless of the growth temperature. Our studies indicate that PsbU, a constituent of the photosystem II complex, protects the oxygen-evolving machinery against heat-induced inactivation.

The PSII complex is a pigment-protein complex that utilizes light energy to drive the transport of electrons and the oxidation of water to oxygen. It is widely recognized that the PSII complex is the most susceptible to heat among various components of the photosynthetic apparatus (Berry and Björkman, 1980; Mamedov et al., 1993). The inactivation of the PSII complex by heat occurs primarily at a catalytic site for the evolution of oxygen, which is composed of a cluster of four Mn atoms (Katoh and San Pietro, 1967; Yamashita and Butler, 1968; Santarius, 1975; Thompson et al., 1989; Mamedov et al., 1993). The loss of two of the four Mn atoms results in the complete loss of oxygen-evolving activity (Nash et al., 1985). Thus, the stabilization against heat of the oxygen-evolving machinery in the PSII complex seems likely to play an essential role in protecting the entire photosynthetic system from heat inactivation.

We have studied the molecular basis of the stabilization of the oxygen-evolving machinery against heat using thylakoid membranes from the cyanobacterium *Synechococcus* sp. PCC 7002. To investigate the factors that are responsible for the heat stability of the oxygen-evolving machinery, which is located on the luminal side of the thylakoid membranes, we disrupted the vesicular structure of the membranes using a low concentration of Triton X-100. This treatment resulted in a remarkable decrease in the heat stability of the oxygen-evolving machinery. Furthermore, heat stability was recovered upon reconstitution of the membranes with the components that had been extracted with Triton X-100. Using a reconstitution assay, we determined that one of the factors responsible for the heat stability of the oxygen-evolving machinery was Cyt c₅₅₉, a Cyt with a low-redox potential (Nishiyama et al., 1994). However, Cyt c₅₅₉ was insufficient to account for the entire restorative ability of the extract obtained from the thylakoid membranes with Triton X-100. This observation implies that other important factors remain to be identified in the extract.

In the present study we purified another protein factor that contributes to the heat stability of the oxygen-evolving machinery from the material that had been extracted from thylakoid membranes with Triton X-100. Nucleotide sequence analysis revealed that the protein obtained was homologous to PsbU, an extrinsic protein of the PSII complex, which has been found in thermophilic cyanobacteria.

**MATERIALS AND METHODS**

**Organism and Culture Conditions**

*Synechococcus* sp. PCC 7002 was obtained from the Culture Collection of the Pasteur Institute (Paris, France). The cells were grown photoautotrophically at 38°C for 3 to 5 d as described previously (Nishiyama et al., 1993).

**Preparation and Treatment of Thylakoid Membranes**

Thylakoid membranes were isolated from cells, as described previously (Nishiyama et al., 1993). The isolated thylakoid membranes were suspended in medium A (50...
pared with that after incubation at 25°C. The increase in the activity of the fraction.

branes was cooled to 25°C and the oxygen-evolving activ-
thylakoid membranes that had been reconstituted with a
given fraction were incubated at 25 and 38°C, and the oxygen-evolving activity after incubation at 38°C was com-
pared with that after incubation at 25°C. The increase in the ratio of activities was regarded as the thermostabilizing activity of the fraction.

Purification of Protein

Thylakoid membranes corresponding to 60 mg of Chl were treated with 0.1% Triton X-100 under the same condi-
tions as described above, with the exception that medium A was replaced by medium B (20 mM Tris-HCl, pH 7.5). The suspension of membranes was then separated into soluble and membrane fractions by centrifugation at 200,000g for 2 h. The soluble fraction was applied to a column (2.6 cm i.d. × 10 cm) of DEAE-Toyopearl 650C (Tosoh, Tokyo, Japan) that had been equilibrated with medium B. The column was washed with 300 mL of me-
dium B to remove Triton X-100 and nonadsorbed compo-
ents. The fractions eluted with medium B that contained 0.5 mM NaCl were collected and dialyzed against 2 L of
medium B for 12 h at 4°C. The dialysate was applied to a column (1.5 cm i.d. × 5 cm) of DEAE-Toyopearl 650S that had been equilibrated with medium B. The column was washed with 50 mL of medium B and was then developed with a linear gradient of NaCl from 0 to 0.3 M in 150 mL of medium B. Active fractions were collected and dialyzed against medium B, as indicated above, and the dialysate was applied to a Mono-Q HR 5/5 column (Pharmacia) that had been equilibrated with medium B. The column was developed with a linear gradient of NaCl from 0 to 0.5 M in 20 mL of medium B. Active fractions were collected and dialyzed for 12 h at 4°C against 1 L of medium E (20 mM Heps-NaOH, pH 7.5, and 10 mM NaCl). The dialysate was concentrated 20-fold using a Centricon-10 centrifugal microconcentrator (Amicon, Beverly, MA).

Analysis of Proteins and Quantitation of Chl

SDS-PAGE was performed as described by Laemmli (1970) on a 10 to 20% gradient polyacrylamide gel. After electrophoresis the gel was stained with 0.1% (w/v) Coo-
massie brilliant blue R-250. For western-blotting analysis, antibodies against the purified 13-kD protein were raised in a rabbit. After SDS-PAGE the proteins on the gel were transferred electrophoretically to a PVDF membrane (Clear Blot Membrane-p, Atto, Tokyo, Japan). The immunochemo-
detector of the protein was performed with the anti-
serum according to the instructions supplied with the Vect-
astain ABC kit (Vector Laboratories, Burlingame, CA). Protein concentrations were determined with a protein assay solution (Bio-Rad) with BSA as the standard (Bradford, 1976). Concentrations of Chl were determined as described by Arnon et al. (1974).

Measurements of Photosynthetic Activity

The photosynthetic evolution of oxygen was measured by monitoring the concentration of oxygen with a Clark-
type oxygen electrode. The activity of PSII in thylakoid membranes was measured at 25°C in the presence of 100 
µM phenyl-1,4-benzoquinone. Red actinic light, at an intensity of 2 mE m⁻² s⁻¹, was provided by an incandescent lamp that was used in conjunction with an HA30 heat-
absorbing optical filter (Hoya, Tokyo, Japan) and an R-60 red optical filter (Toshiba, Tokyo, Japan).

Analysis of the Amino Acid Composition and
Amino-Terminal Amino Acid Sequence

The purified protein was hydrolyzed in 5 N HCl at 110°C for 24 or 72 h, and the amino acid composition was deter-
mined with an automated amino acid analyzer (model 835, Hitachi, Tokyo, Japan). The amino-terminal sequence was determined by automated Edman degradation with a pro-
tein sequence analyzer (model 492, Applied Biosystems).

Molecular Cloning

The sequence of 58 amino acids at the amino-terminal end of the purified protein was determined. A DNA frag-
ment corresponding to the 58 amino acid residues was ampli-
fied by PCR as follows: Genomic DNA was isolated from Synechococcus sp. PCC 7002 cells as described by Williams (1988), and it was used as the template for PCR. Degenerate primers GA(GA)CG(CG)GT(GTC)AA(TC)-
CCCG(CT)GA(TC)AAAGT (amino acid residues 1 to 9 of the amino-terminal sequence of the protein) and GG-
(GAT)AT(AG)TT(CAG)A(GA)(GAC)AC(AG)TC(TC)TC-
(GAC)AC (amino acid residues 58 to 51) were used as forward and reverse primers, respectively. The product of
the PCR of 190 bp was cloned into the plasmid vector pT7Blue (Novagen, Madison, WI), and the nucleotide sequence of the cloned DNA was determined to ensure that the cloned gene encoded a portion of the purified protein.

A genomic DNA library of Synechococcus sp. PCC 7002 was constructed in the phage vector ADASHII (Stratagene), and it was screened with the radioactively labeled probe of 190 bp. Recombinant phage DNA was isolated from the four phages that gave a positive signal and was subjected to restriction mapping and Southern-blotting analysis. A DNA fragment of 2.3 kb, which contained a full-size psbU gene, was obtained by digestion with HindIII. It was subcloned into the HindIII site of pUC19 (Pharmacia), and the resultant plasmid was designated pUH239. The nucleotide sequence of a 649-bp fragment within the insert of pUH239 was determined with an automated DNA sequencer (model 373A, Applied Biosystems).

RESULTS

Purification and Identification of a Protein That Contributes to the Heat Stability of the Oxygen-Evolving Machinery

We reported previously that disruption of the vesicular structure of thylakoid membranes by treatment with 0.1% Triton X-100 resulted in a marked decrease in the heat stability of the oxygen-evolving machinery and that the heat stability could be restored when the membranes were reconstituted with the components that had been extracted by this treatment (Nishiyama et al., 1994).

The activity in the extract that was capable of restoring heat stability was recovered in fractions that were eluted in 0.5 M NaCl from the first anion-exchange column (DEAE-Toyopearl 650C). When the active fractions were subjected to a second round of anion-exchange chromatography on DEAE-Toyopearl 650S, the activity was separated into three peaks upon the elution with a linear gradient of NaCl (Fig. 1). The first peak of activity was assigned to Cyt c550, which had been identified as one of the factors that contribute to the heat stability of the oxygen-evolving machinery (Nishiyama et al., 1994). The material in the second peak was investigated in the present study.

We purified a protein that was responsible for the second peak of activity by anion-exchange chromatography on a Mono-Q HR 5/5 column and subsequent chromatofocusing on a Mono-P HR 5/5 column. The fractions obtained from the Mono-Q column that were able to restore heat stability were eluted between 0.2 and 0.3 M NaCl. These fractions included a trace of Cyt c550, which was, as shown previously, able to increase heat stability. During chromatography on the Mono-P column most of the proteins were eluted between pH 5.5 and 4.0. No relevant activity was detected, except for the residual Cyt c550, which was eluted at pH 4.0. Activity was, however, recovered as a single peak when the column was subsequently developed with a gradient of NaCl after re-equilibration at pH 5.5 (Fig. 2). This elution property suggested that the functional protein might have a pI of less than 4.0.

Figure 1. Results of the chromatography on the second anion-exchange column (DEAE-Toyopearl 650S) of the active fractions that were obtained after the anion-exchange chromatography on the first column. Activity of individual fractions was assayed as described in "Materials and Methods."

Figure 3 shows the protein profiles obtained after SDS-gel electrophoresis of the active fractions from each step in the purification. The active fraction obtained from the Mono-P column contained a single protein of 13 kD, without any contaminants.

The sequence of 58 amino acids at the amino-terminal end of the purified protein was determined. A homology search revealed that the protein was homologous to PsbU, which was formerly known as the 9- or 12-kD extrinsic protein of the cyanobacterial PSII complex. This protein has been found in the thermophilic cyanobacteria Phormidium laminosum (Stewart et al., 1985a) and Synechococcus vulcanus (Shen et al., 1992) and in the red alga Cyanidium caldarium (Enami et al., 1995). Thus, we identified the 13-kD protein as PsbU of Synechococcus sp. PCC 7002.

Molecular Cloning of the psbU Gene

A genomic DNA library of Synechococcus sp. PCC 7002 was constructed in the phage vector λDASHII and it was screened with the 190-bp probe, obtained by amplification of a portion of the psbU gene by PCR. The recombinant phage DNA was isolated from four plaques that gave a positive hybridization signal. The restriction maps of the isolated DNAs allowed us to select a 2.3-kb HindIII fragment that contained a full-size psbU gene. We subcloned this fragment into the HindIII site of pUC19 and sequenced a 649-bp portion of the fragment. An open-reading frame of
414 bp, which encoded PsbU, was located within the sequenced fragment (Fig. 4). The deduced amino acid sequence indicates that the product of the open-reading frame consists of a transit peptide of 42 residues and a mature protein of 96 residues. The deduced amino acid composition of the mature protein was identical to that of the purified protein, confirming the identity of the gene. The presence of a transit peptide suggests that PsbU is located on the luminal side of thylakoid membranes.

Thermoprotective Role of PsbU

Figure 5A shows the effects of the addition of purified PsbU on the temperature profile of heat inactivation of the oxygen-evolving machinery in thylakoid membranes that had been treated with 0.1% Triton X-100. The protein was added to a suspension of thylakoid membranes with a Chl concentration of 5 μg mL⁻¹ to a final concentration of 5 μg mL⁻¹. The addition of the protein resulted in a marked increase in the heat stability of the membranes, with the temperature for 50% inactivation shifting from 37 to 41°C. In terms of the temperature for 50% inactivation, heat stability appeared to return to the initial level upon the addition of PsbU. However, at higher temperatures the restoration of heat stability was less effective. A similar profile of restoration was observed when only Cyt c₅₅₀ was added to thylakoid membranes that had been treated with Triton X-100 (Nishiyama et al., 1994), suggesting that neither PsbU nor Cyt c₅₅₀ alone is sufficient for full restoration. To compare the thermoprotective activity of PsbU with that of Cyt c₅₅₀, we also examined the effects on heat stability of Cyt c₅₅₀ alone and in combination with PsbU. The addition of Cyt c₅₅₀ alone yielded a profile of increasing heat stability similar to that obtained with PsbU, but the restorative effect of Cyt c₅₅₀ was approximately 40% smaller than that of PsbU. The combination of PsbU and Cyt c₅₅₀ had a greater effect on heat stability than did PsbU alone. Thus, we can infer that, although PsbU and Cyt c₅₅₀ act independently to stabilize the oxygen-evolving machinery against heat, both proteins are necessary for construction of the thermally stable PSII complex.
AAG CTT GAT ATT TTA CAT AAA TCT AAA AAT CAT CAA AAA AAG CTG GGA
AAA
from Synechococcus
ATG GGG CTC GTC CTC ATG TTC GGT TGC GCG TTC TTT AGC GTT CAG CCC
high temperature, we compared the levels of expression of
analysis. The amounts of PsbU from cells grown at
PsbU in cells grown at different temperatures by western
sequence that was determined directly from the protein is underlined.
Figure 4. letter code under the nucleotide sequence. The amino-terminal se-
AAG
CAA GCC CAA GCC CTC GAT TTA AGC AAT GGC TTT GTT TCT GCA GCC GTG
192
TCG ATA AAA CTG AGG TAC CCA GCC G
649
CTC GGT GAG CGT GTT AAT CCC GCT GAC AAG GTG CTT GAG TCA GAA TAC
240
LGERVNPADKVLESEY
56*
GCC AAG AAA ATC GAC CTA AAG AAC GGC GTC GCT CTT TCC GGC GAG
288
GKRLDQOLMNASVRLFEE
72*
CTC GGT GSA TTT TAT CCC ACA TTA GCT GCA AAC GCA ATC GCA AAA GCA
336
LQYVFTSRRTRITGFA
66*
GCC TAG GAA GAT GGT GAA GCA AGT GAC CTC GAT GAA AAA GCA ATG ACC
384
PVDQEDVHELRTDLR SE
104*
AAG CAA CTC GCA GCA TTA GAG ACA ATG TTG GAG GCT TGC ACT GTA ACA
432
KQLARLERENLRFITVT
120*
CCG CTT GAT GCT GCT TTT ATT GTA GCT GAC CAA GCT CTC ACC GGC GGT
490
PPADVPIGDSGDOQLNTGC
236*
GAC TAT TAA AAA TAC AAA TAA GTC ATT GAT GAG ATT GAG AAG ATT TTT
498
D1
138*
GTA AGT CAA ACC TAA ATT TCA ATT TCG TAA ATT GAT GAG GCA
576
TAG GCT TTA TTA CCA CAA TTT TTA GAA GAT GGC GGG GSG TGG TGC GGA TAG
624
TGG ATA AAA CGG TAG ACC GCC G
649
Figure 4. The nucleotide sequence of the gene for the 13-kD protein from Synechococcus sp. PCC 7002 (GenBank accession no. X93509). The deduced amino acid sequence is shown in the one-letter code under the nucleotide sequence. The amino-terminal sequence that was determined directly from the protein is underlined. A putative ribosome-binding site is double-underlined.

Expression of PsbU
To examine whether the synthesis of PsbU is induced by high temperature, we compared the levels of expression of PsbU in cells grown at different temperatures by western analysis. The amounts of PsbU from cells grown at 25 and 38°C were the same in total cell extract, as well as in thylakoid membranes (Fig. 6). These observations suggest that PsbU was not induced by high temperature. We also examined whether posttranslational modification might occur in response to high temperature. PsbU proteins were purified from cells grown at 25 and 38°C, and they were compared in terms of molecular size, amino acid composition, and pl. All of the examined properties of both proteins were the same (data not shown). Thus, we concluded that PsbU is expressed constitutively and is localized in thylakoid membranes without any modification when growth temperature is increased.

DISCUSSION
In the present study we identified a protein that is involved in the stabilization against heat of the oxygen-evolving machinery in Synechococcus sp. PCC 7002. We had assumed initially that factors that contribute to the heat stability of the oxygen-evolving system would be located near the side of evolution of oxygen, namely on the luminal side of thylakoid membranes. Disruption of the vesicular structure of thylakoid membranes with a low concentration of Triton X-100 resulted in a marked decrease in the heat stability of the oxygen-evolving machinery with a concomitant release of protein components from the membranes. The released components were capable of restoring heat stability when returned to the preparation of thylakoid membranes, an indication of their involvement in heat stability (Nishiyama et al., 1994). In the present study, using a reconstitution assay, we purified a 13-kD protein that was able to restore heat stability. The amino acid sequence deduced from the nucleotide sequence indicated that the 13-kD protein was homologous to PsbU, an extrin-

Figure 5. The effect of PsbU on the heat stability of the oxygen-evolving machinery of PSII. A, Purified PsbU was added to thylakoid membranes that had been treated with 0.1% Triton X-100 at a final Chl:protein ratio of 1:1 (w/w). The membranes were then incubated at the designated temperature for 20 min in darkness. Oxygen evolution was measured at 25°C in the presence of phenyl-1,4-benzoquinone. O, No treatment with Triton X-100; □, after treatment with Triton X-100; ●, after treatment with Triton X-100 and addition of PsbU. The oxygen-evolving activities taken as 100% were 185, 170, and 180 μmol O₂ mg⁻¹ h⁻¹, respectively. B, Various amounts of PsbU and Cyt c₅₅₀ were added to thylakoid membranes that had been treated with 0.1% Triton X-100. Heat stability is presented as the oxygen-evolving activity after incubation at 38°C relative to that after incubation at 25°C for 20 min. O, No treatment with Triton X-100; △, treatment with Triton X-100 and addition of Cyt c₅₅₀; ●, treatment with Triton X-100 and addition of PsbU; and ▲, treatment with Triton X-100 and addition of PsbU plus Cyt c₅₅₀.
PsbU was first identified as a 9-kD protein that was associated with the PSII complex from the thermophilic cyanobacterium *P. laminosum* (Stewart et al., 1985a). It was subsequently proposed that the 9-kD protein was bound to the donor side of PSII and was essential for the oxygen-evolving activity, in view of the observation that the release of the 9-kD protein from the PSII complex of *P. laminosum* was closely correlated with a decrease in oxygen-evolving activity (Stewart et al., 1985b; Stamatakis and Papageorgiou, 1993). This conclusion was supported by the results of experiments that involved the dissociation from and readdition to the PSII complex of the 9-kD protein of *P. laminosum* (Rolf and Bendall, 1989). In addition, the proposed localization of the 9-kD protein that had been deduced from the above biochemical evidence was reinforced by the finding of a leader sequence in the gene for this protein, which included a domain typical of proteins that are targeted to the thylakoid lumen (Wallace et al., 1989).

A homolog of the 9-kD protein was found in another thermophilic cyanobacterium, *Synechococcus vulcanus*, as a 12-kD protein that was associated stoichiometrically with the PSII complex (Shen et al., 1992). The 12-kD protein was located exclusively in the PSII complex (Shen and Inoue, 1993a), and it appeared to regulate the oxygen-evolving activity in cooperation with Cyt c550, another extrinsic protein of the PSII complex in *S. vulcanus* (Shen and Inoue, 1993b). These PsbU proteins, as well as Cyt c550, are not found in higher plants. In turn, the 18- and 23-kD extrinsic proteins of the PSII complex of higher plants are not found in cyanobacteria (Stewart et al., 1985a; Shen and Inoue, 1993a). The similarities in terms of binding and functional properties of PsbU and Cyt c550 to the 18- and 23-kD proteins of higher plants may imply an evolutionary connection among the extrinsic proteins of the PSII complex (Shen et al., 1993b). It is of interest that the red alga *Cyanidium caldarium* has both PsbU and Cyt c550 (Enami et al., 1995).

We compared the amino acid sequence of PsbU from *Synechococcus* sp. PCC 7002 with sequences of PsbU from other cyanobacteria (Fig. 7). The sequence of PsbU from *Synechococcus* sp. PCC 7002 is 42% homologous to the sequence of PsbU from *P. laminosum* (Wallace et al., 1989) and 48% homologous to the sequence of PsbU from *Synechocystis* sp. PCC 6803 (Kaneko et al., 1996).

Compared with the PsbU proteins from the thermophilic cyanobacterium, PsbU from *Synechococcus* sp. PCC 7002, a mesophilic cyanobacterium, exhibited distinct differences in terms of binding and functional properties. First, the release of PsbU from the PSII complex of *P. laminosum* required treatment with 1 M chloride salts, such as NaCl, CaCl2, or MgCl2, 0.8 M alkaline Tris, or a reduction of concentration of glycerol in the medium (Stewart et al., 1985a), whereas 1 M CaCl2, but neither NaCl nor a low-glycerol medium, released PsbU from the PSII complex in *S. vulcanus* (Shen et al., 1992). By contrast, PsbU of *Synechococcus* sp. PCC 7002 was released from the PSII complex upon disruption of the vesicular structure of thylakoid membranes with a low concentration of Triton X-100 or by ultrasonic oscillation (Y. Nishiyama, D.A. Los, and N. Mu-rakami, unpublished data). Western analysis revealed that

![Figure 6](image-url)
PsbU was also present in a soluble fraction that had been separated from the membranes after breakage of cells with glass beads, suggesting that a portion of the protein might be released when thylakoid vesicles are disrupted mechan-
ically (Y. Nishiyama, D.A. Los, and N. Murata, unpublished data). Therefore, PsbU of Synechococcus sp. PCC 7002 seems likely to be more loosely associated with the PSI complex than is the case in the thermophilic species.

Second, in the thermophilic species PsbU is essential for oxygen-evolving activity (Rolf and Bendall, 1989; Shen and Inoue, 1993b). By contrast, in Synechococcus sp. PCC 7002, neither the dissociation of PsbU from thylakoid membranes with Triton X-100 nor the reconstitution of membranes with PsbU affected the oxygen-evolving activity to any significant extent. This observation indicates that PsbU is not essential for the oxygen-evolving activity in Synechococcus sp. PCC 7002. A similar difference in terms of the binding and functional characteristics of Cyt c550 was observed when the thermophilic species and Synechococcus sp. PCC 7002 were compared. Thus, it appears that PsbU and Cyt c550 play a protective role, without which the PSI complex in Synechococcus sp. PCC 7002 is susceptible to inactivation by heat. We can speculate that the thermophilic species evolved a more stable oxygen-evolving machinery, to withstand the extremely high temperatures of their natural habitats, by strengthening the association of these extrinsic proteins with the PSI complex, where these proteins participate in the catalytic activity required for the evolution of oxygen.

When the cells of Synechococcus sp. PCC 7002 acclimate to high temperature, the heat stability of their photosynthetic machinery is enhanced (Nishiyama et al., 1993). This acclimation response has been observed in a number of higher plants and cyanobacteria (Berry and Björkman, 1980; Lehel et al., 1993), although the molecular mechanism underlying such acclimation remains to be clarified. We have found that the oxygen-evolving machinery, which is the most sensitive to heat of all components of the photosynthetic system, is stabilized upon acclimation to high temperature (Nishiyama et al., 1993). Therefore, we should now investigate any factor that contributes to the stabilization of the oxygen-evolving machinery involved in this acclimation response.

Our findings related to the mode of expression of PsbU demonstrate that this protein is expressed constitutively and is localized in the thylakoid membranes, without any modifications, during acclimation to high temperature. These observations suggest that PsbU is one of the macro-
molecules that constitutively stabilize the oxygen-evolving machinery and that it is not a factor that directly modifies the heat stability of the oxygen-evolving machinery during the acclimation to high temperature. However, the dramatic effect of PsbU, as well as of Cyt c550, on heat stability suggests that these extrinsic proteins are indispensable for the construction of a thermally stable PSI complex. It remains to be determined whether these extrinsic proteins are involved in the mechanism that underlies the enhancement of the heat stability of the oxygen-evolving machinery, which occurs during acclimation to high temperature.

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