Analysis of the psbU Gene Encoding the 12-kDa Extrinsic Protein of Photosystem II and Studies on Its Role by Deletion Mutagenesis in Synechocystis sp. PCC 6803*

(Received for publication, March 17, 1997, and in revised form, May 6, 1997)

Jian-Ren Shen‡, Masahiko Ikeuchi§, and Yorinao Inoue

From the Solar Energy Research Group and Photosynthesis Research Laboratory, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan and the §Department of Biology, University of Tokyo, Komaba, Tokyo 153, Japan

The gene encoding the 12-kDa extrinsic protein of photosystem II from Synechocystis sp. PCC 6803 was cloned based on N-terminal sequence of the mature protein. This gene, named psbU, encodes a polypeptide of 131 residues, the first 36 residues of which were absent in the mature protein and thus served as a transit peptide required for its transport into the thylakoid lumen. A psbU gene deletion mutant grew photoautotrophically in normal BG11 medium at almost the same rate as that of the wild type strain. This mutant, however, grew apparently slower than the wild type did upon depletion of Ca²⁺ or Cl⁻ from the growth medium. Photosystem II oxygen evolution decreased to 81% in the mutant as compared with that in the wild type, and the thermoluminescence B- and Q-bands shifted to higher temperatures accompanied by an increase in the Q-band intensity. These results indicate that the 12-kDa protein is not essential for oxygen evolution but may play a role in optimizing the ion (Ca²⁺ and Cl⁻) environment and maintaining a functional structure of the cyanobacterial oxygen-evolving complex. In addition, a double deletion mutant lacking cytochrome c-550 and the 12-kDa protein grew photoautotrophically with a phenotype identical to that of the single deletion mutant of cytochrome c-550. This supports our previous biochemical results that the 12-kDa protein cannot bind to photosystem II in the absence of cytochrome c-550 (Shen, J.-R., and Inoue, Y. (1993) Biochemistry 32, 1825-1832).

The oxygen-evolving system of cyanobacteria contains three extrinsic proteins, namely, a 33-kDa protein, cytochrome c-550, and a 12-kDa protein. The genes coding for the 33-kDa protein and cytochrome c-550 are psbO and psbV genes, respectively (for reviews see Refs. 1 and 2), whereas the gene for the 12-kDa protein has been tentatively named psbU (3, 4). The 33-kDa protein is commonly found in higher plant and cyanobacterial PSII, and its function has been studied extensively by both in vitro biochemical approaches and in vivo mutagenesis studies. Results from these studies suggested that the 33-kDa protein plays an important role in stabilizing the tetramanganese cluster, which directly catalyzes the water-splitting reaction. Loss of this protein by biochemical removal from isolated PSII (5-7) or genetic deletion (8, 9) from algal cells leads to a significant loss of the oxygen-evolving activity and in some conditions loss of manganese atoms from the tetramanganese cluster. The other two proteins, cytochrome c-550 and the 12-kDa protein, however, are present only in algal-type PSII but absent in higher plant PSII. Our previous in vitro biochemical (10) and in vivo genetic studies (11) have indicated that cytochrome c-550 is required for maintaining both the oxygen evolution and PSII stability in vivo. This cytochrome can bind to PSII essentially independent of the other extrinsic proteins (10). In accordance with this, a double deletion mutant of Synechocystis sp. PCC 6803 lacking both the 33-kDa protein and cytochrome c-550 showed a complete loss of photoautotrophic growth, which is caused by deactivation of oxygen evolution and destabilization of PSII in vivo (12). In contrast, both the single deletion mutant of the 33-kDa protein (9) or cytochrome c-550 (11) were able to grow photoautotrophically, albeit with reduced rates. These studies suggested that cytochrome c-550 binds to and functions in cyanobacterial PSII independent of the 33-kDa protein in maintaining the oxygen-evolving activity and PSII stability (12).

The 12-kDa protein was first found as a 9-kDa protein in PSII purified from a thermophilic Phormidium laminosum (13, 14). Dissociation of this protein from the isolated PSII caused a decrease in oxygen evolution that was partially restored upon rebinding of the protein (14, 15). The P. laminosum gene encoding the protein has been cloned and includes a leader sequence required for its transport into the thylakoid lumen (16). We have confirmed the presence of a homologous 12-kDa protein in PSII purified from another thermophilic cyanobacterium, Synechococcus vulcanus (17). Lack of the 12-kDa protein also led to a decrease in oxygen evolution of the Synechococcus PSII that was restored by rebinding of the protein (10). We further showed that binding of the 12-kDa protein in the isolated Synechococcus PSII requires presence of both the 33-kDa protein and cytochrome c-550; in the absence of either of the two proteins, the 12-kDa protein cannot bind to PSII efficiently (10). These results suggested that the 12-kDa protein is in close contact with both the 33-kDa protein and cytochrome c-550 at the lumenal side of cyanobacterial PSII; this has been confirmed by in vitro cross-linking studies showing that all of the three proteins were cross-linked together by 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (18). These studies were carried out in vitro with PSII purified from thermophilic cyanobacteria. In the present work, we demonstrated...
the presence of the 12-kDa protein in thylakoids of the mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803, was grown in BG11 medium at 30 °C at a light intensity of 25–30 einsteins m⁻² s⁻¹. Thylakoid membranes were isolated from the *Synechocystis* cells by glass beads according to Ref. 19, and the isolated thylakoids were treated by sonication to release extrinsic proteins as described in Ref. 20. The obtained sonication extract was separated by a Mono Q column using a fast protein liquid chromatography system (Pharmacia Biotech Inc.) (11). The fractions from column chromatography were collected and electrophoresed in a gel containing 16% polyacrylamide and 6.0 M urea, and protein bands around the 12-kDa molecular mass region were transferred to a polyvinylidene difluoride membrane followed by N-terminal amino acid sequencing. The 12-kDa protein was identified by comparing the obtained sequences with the 9-kDa protein sequence from *S. vulcanus*. Based on the N-terminal sequence, the gene coding for the 12-kDa protein was cloned from genomic DNA of *S. vulcanus* by N-terminal coding region sequencing method. PCR reaction was carried out using an Expand High Fidelity PCR kit (Boehringer Mannheim), and each PCR product was sequenced from three independent clones to eliminate any possible inconsistencies. To obtain the clone containing the complete *psbU* gene, another PCR reaction was carried out with genomic DNA as template and primers flanking the whole gene. The resulted PCR product was cloned into pUC119 and used for subsequent mutagenesis studies.

To delete the 12-kDa protein from *Synechocystis* PCC 6803, a plasmid was constructed in which a 0.45-kb EcoT14 I-XbaI fragment containing the entire coding region of the gene was replaced by a kanamycin-resistant cassette. This plasmid was used to transform the glucose-tolerant *Synechocystis* PCC 6803 strain. Cyanobacterial transformation and selection of the kanamycin-resistant transformant were carried out according to published procedures (21). As a control, another plasmid was constructed in which the kanamycin-resistant cassette was inserted into an XbaI site downstream of the coding region of the 12-kDa protein gene, and this plasmid was also transformed to *Synechocystis* PCC 6803.

A double deletion mutant lacking cytochrome c-550 and the 12-kDa protein was constructed by introducing the *psbU* gene deletion plasmid into a host cell in which the *psbU* gene coding for cytochrome c-550 has been replaced by an erythromycin-resistant cassette as described previously (11). The transformants were selected and propagated in BG11 plates containing 25 μg atrazine, 5 μM glucose, 10 μg kanamycin/ml, and 20 μg/ml erythromycin.

Growth curves of the wild type and mutant strains in liquid BG11 were recorded by measuring the light scattering of cells at 730 nm. For depletion of Ca²⁺ or Cl⁻ from the BG11 medium, 0.24 mM CaCl₂ in the original medium was replaced by either 0.48 mM NaCl or 0.24 mM Ca(NO₃)₂. *Synechocystis* cells grown in their mid-logarithmic phase were harvested, washed twice with distilled water, and then transferred to the growth medium lacking Ca²⁺ or Cl⁻. For photoreplication, harvested cells were broken by glass beads (100–150 μm), solubilized, and then applied to a 16–22% polyacrylamide gradient gel containing 6.0 M urea. Cytochromes of c-type were detected by monitoring peroxidase activity of the hemes on the gel with 3,3',5,5'-tetrachloroazidine and H₂O₂ as described in Refs. 20 and 22.

Oxygen evolution of the *Synechocystis* cells was measured with a Clark-type oxygen electrode under continuous, saturating yellow light at 30 °C in BG11 with 0.6 mM 2,6-dichlorobenzoquinone and 1 mM potassium ferricyanide as acceptors. For thermoluminescence measurement, harvested *Synechocystis* cells were suspended in BG11 in the absence or the presence of 20 μg DCMU at 100 μg chlorophyll/ml, adapted in the dark for 5 min at room temperature, and then illuminated with single turn-over flashes at 0 °C for samples without DCMU or with continuous light for 30 s at −5 °C for samples supplemented with DCMU. Thermoluminescence glow curves were recorded as described in Ref. 23, with a heating rate of 1.0 °C/s.

**RESULTS**

**Cloning and Sequence Analysis of the *psbU* Gene Coding for the 12-kDa Protein**—The 12-kDa protein was identified by N-terminal sequencing of several protein bands around 12-kDa molecular mass region obtained by column chromatography of sonication extracts from thylakoid membranes of *Synechocystis* PCC 6803. The sequence obtained was ELNAVDAKLTTDF-1298 in the DNA fragment shown in Fig. 1, because this codon is homologous to the 9-kDa protein of *P. linalosum* and the 12-kDa protein of *S. vulcanus*.
preceded by a typical ribosome-binding sequence (Shine-Dalgarno sequence, AGGAG, underlined in Fig. 1) 7 base pairs upstream, as well as a stop codon 39 base pairs upstream in the same reading frame (indicated by an asterisk in Fig. 1). According to this assignment, the gene encodes a polypeptide of 131 amino acid residues with a total molecular mass of 14231 Da. Hydropathy analysis indicated that the derived amino acid sequence is mostly hydrophilic, except the first 36 residues, which form a major hydrophobic loop (not shown). This is in agreement with the previous results that the 12-kDa protein is a hydrophilic one that is associated with lumenal surface of the thylakoid membranes (10, 13–17). As upon PCR amplification of the psbU gene, we detected only one band of the gene, we consider that there is only one copy of the gene in Synechocystis PCC 6803. This is consistent with the results from computer analysis of the complete genome sequence of Synechocystis PCC 6803 that was recently determined (24).

The N-terminal sequence determined for the mature 12-kDa protein corresponds to the sequence starting from residue number 37 of the gene-derived amino acid sequence. This indicates that the cleavage of the first 36 residues after synthesis of the protein, which gave rise to a mature 12-kDa protein of 95 residues with a calculated molecular mass of 10490 Da. Thus, the first 36 residues served as a transit peptide for the protein to be transferred across membranes. This is consistent with the previous results that the 12-kDa protein is associated with the lumenal side of thylakoid membranes (10). In fact, a similar transit peptide has been found for the homologous 9-kDa protein from *P. laminosum* (16), and recently it has been reported that the same gene from *Synechococcus* sp. PCC 7002 also carried a transit peptide (5). Although the three transit peptides for the 12-kDa protein have virtually no similarity in their primary sequences, they share some common features of bacterial-type transit peptides (25), e.g., there is a positive charge in the N terminus and a hydrophobic cluster in the middle, and the residue immediately before the cleavage site is Ala in all the three sequences (Fig. 2). Fig. 2 compared the gene-derived 12-kDa protein sequence from *Synechocystis* PCC 6803 with those from *Synechococcus* sp. PCC 7002 (3) and *P. laminosum* (16) and also with the N-terminal partial sequence from *S. vulcanus* (17) and a red alga, *Cyanidium caldarium* (26). Among the three cyanobacteria for which the psbU gene has been cloned, an overall identity of 48.5–56.3% can be seen for the mature part of the sequence. When this comparison was made for the first 65 residues for which the sequence from *S. vulcanus* is known, the total homology lies between 49.2–57.1% among the four species of cyanobacteria compared. The sequence homology in the same N-terminal region, however, are only 30.3–36.5% between cyanobacteria and the red alga *C. caldarium*; this is significantly lower than those observed among different species of cyanobacteria. This indicates a remarkably higher divergence in the 12-kDa protein sequence between cyanobacteria and red algae than those found among cyanobacteria.

**Mutant Constructions**—A deletion mutant ΔpsbU, was constructed by replacing a 0.45-kb EcoT14I-XbaI fragment containing the whole psbU gene with a 1.3-kb kanamycin-resistant cassette. This replacement was confirmed by PCR amplification of the DNA region containing the psbU gene. As shown in Fig. 3, although PCR amplification with two primers whose sequences correspond to the 5'- and 3'-sequences of the DNA fragment shown in Fig. 1 yielded a fragment of 0.92 kb from the wild type cells, the same amplification yielded a fragment of 1.8 kb from the targeted 12-kDa protein deletion mutant, which is exactly the same as would be expected if the 0.45-kb psbU gene-containing fragment was replaced by the 1.3-kb kanamycin cassette. The original 0.92-kb fragment disappeared completely, indicating a successful deletion of the only psbU gene in the mutant. Fig. 3 also shows the successful construction of a double deletion mutant, ΔpsbU/ΔpsbV, which is depleted of both the 12-kDa protein and cytochrome c-550. In this mutant, the psbU gene was replaced by the kanamycin cassette (Fig. 3, *lane 4 in panel A*) and a large part of the psbV gene was replaced by an erythromycin-resistant cassette (11) (Fig. 3, *lane 4 in panel B*). As a control, a *Synechocystis* strain designated psbU/Km was constructed by inserting the 1.3-kb kanamycin cassette into the XbaI site downstream of the psbU gene. This insertion does not inactivate the psbU gene and gives rise to a PCR fragment of 2.2 kb as shown in *lane 2 of panel A* in Fig. 3, which thus indicates a successful construction of the psbU/Km strain.

**Characterization of the Mutants**—Fig. 4 shows the photautotrophic growth curves and oxygen evolution of wild type and mutant strains. Both the deletion mutant ΔpsbU and the control strain psbU/Km showed a growth rate close to that of the wild type, indicating that deletion of the 12-kDa protein had very little, if any, effect on growth of the *Synechocystis* cells. The PSII oxygen evolution decreased to 81% in the ΔpsbU mutant as compared with that of the wild type strain. In contrast, the psbU/Km strain had the same oxygen-evolving activity as that of the wild type. These results indicate a slight decrease in the oxygen evolution upon deletion of the psbU gene.
Analysis and Deletion Mutagenesis of the psbU Gene

PSII activity showed a smaller decrease in their growth rates or overall photosynthetic electron transport activities than the decrease observed in the PSII oxygen-evolving activity (see for example Ref. 9).

The growth rate of the double deletion mutant ΔpsbU/ΔpsbV decreased to half of the wild type strain (Fig. 4); this is comparable with that of the single deletion mutant ΔpsbV reported previously (11). The activity of the double deletion mutant, ΔpsbU/ΔpsbV, is 41% of the wild type strain (Fig. 4), which is also similar to the activity observed for the single deletion mutant, ΔpsbU (11). These results are in contrast to the results observed with the single deletion mutant ΔpsbU and indicates that further deletion of the psbU gene from the ΔpsbV mutant strain had no effect on its growth and oxygen evolution. This in turn suggests that the 12-kDa protein did not function in oxygen evolution of the ΔpsbV mutant, in agreement with the previous in vitro results that the 12-kDa protein cannot bind to and function in cyanobacterial PSII in the absence of both cytochrome c-550 and the 33-kDa protein (10).

The effect of deletion of the 12-kDa protein on growth was also examined in the absence of Ca\textsuperscript{2+} or Cl\textsuperscript{-} in the growth medium. As shown in Fig. 5, the wild type strain was able to grow in the medium depleted of either Ca\textsuperscript{2+} or Cl\textsuperscript{-} with a slightly reduced rate in agreement with the results reported previously (27, 28). A similar growth was observed for the strain psb\textsuperscript{U/Km}. The deletion mutant ΔpsbU, however, showed an apparent decrease in the growth rate in the absence of either Ca\textsuperscript{2+} or Cl\textsuperscript{-}. This is different from the growth in the normal BG11 medium, where no effect was seen upon deletion of the gene (Fig. 4). This suggests a role of the 12-kDa protein in maintaining the optimum ion (Ca\textsuperscript{2+} and Cl\textsuperscript{-}) environment required for cyanobacterial oxygen evolution. Presumably, deletion of the 12-kDa protein decreased the affinity of PSII for Ca\textsuperscript{2+} and Cl\textsuperscript{-}, which then leads to a reduction of the growth in the absence of one of these two ions. In contrast, the double deletion mutant ΔpsbU/ΔpsbV cannot grow at all in the absence of either Ca\textsuperscript{2+} or Cl\textsuperscript{-}. This is caused primarily by deletion of the psbV gene, because the single deletion mutant ΔpsbV is already unable to grow in the absence of Ca\textsuperscript{2+} or Cl\textsuperscript{-}.

The oxygen-evolving system in the mutant strains was further investigated by thermoluminescence from cells excited with single turn-over flashes. The thermoluminescence B-band, which is obtained in the absence of DCMU and arises principally from charge recombination between S\textsubscript{2} and Q\textsubscript{B}, had a peak temperature of 24°C for the wild type strain (Fig. 6). Its intensity becomes larger following two flashes of illumination than after one flash of illumination, a typical feature observed for thermoluminescence from whole cyanobacterial cells (30). The peak temperature and intensity of the B-band from the control strain psb\textsuperscript{U/Km} is very similar to those of the wild type strain. The ΔpsbU strain also showed a similar intensity of the B-band as that of the wild type or psb\textsuperscript{U/Km} strains following either one, two, or three flashes. Its peak temperature, however, shifted to 28°C; this is apparently higher than the peak temperature of the B-band from wild type or psb\textsuperscript{U/Km} strains. This suggests a possible modification of the S\textsubscript{2}-state upon deletion of the psbU gene. The peak temperature of B-band from the double deletion mutant ΔpsbU/ΔpsbV is around 30°C, which is close to the single deletion mutant ΔpsbU. The intensity of the B-band from the double deletion mutant ΔpsbU/ΔpsbV, however, decreased significantly than that of the wild type or psb\textsuperscript{U/Km} strains. This implies that the shift of the B-band peak temperature is caused by deletion of

---

2 J.-R. Shen, M. Qian, Y. Inoue, and R. L. Burnap, manuscript in preparation.
The 12-kDa protein, whereas deletion of cytochrome c-550 mainly affected the overall activity of PSII oxygen evolution but had essentially no effects on properties of any single S-states.

The thermoluminescence Q-band, which is obtained in the presence of DCMU and arises from charge recombination of S2QA and S2QA', showed a peak temperature of 18 °C for the wild type and psbU/Km strains. This temperature is slightly higher than that reported previously for cyanobacterial cells (30), presumably because of the difference in the growth conditions between the present and previous studies. The intensity of the Q-band is significantly higher than the intensity of the B-band from the same strains. The Q-band from the ΔpsbU mutant strain showed a peak temperature of 22 °C, which is higher than the Q-band from the wild type or psbU/Km strains. This resembles the situation observed for the B-band, thus confirming the modification of the ΔpsbU mutant. These features are also observed in the single deletion mutant ΔpsbV, suggesting that the 12-kDa protein already lost its function in the ΔpsbV mutant. The smaller increase in the intensity of the Q-band in the ΔpsbU/ΔpsbV mutant is apparently due to the fact that the number of functional PSII reaction centers is decreased in the double deletion mutant, because deletion of psbV has been shown to destabilize the PSII complex (11).

**DISCUSSION**

**Features of the psbU Gene**—The psbU gene cloned in the present study represents the first gene from mesophilic cyanobacteria coding for the 12-kDa extrinsic protein of algal PSII. As in the thermophilic cyanobacteria (16), the gene obtained here had a leader sequence typical of a prokaryotic type. This suggests the transport of the protein after its synthesis, in support of the previous biochemical evidence that this protein is located in the lumenal side of the thylakoid membrane (10).

The 12-kDa protein, together with cytochrome c-550, are two PSII extrinsic proteins first found in prokaryotic algae cyanobacteria (17), and they recently have been confirmed in a eukaryotic red alga C. caldarium (33). The psbV gene for cytochrome c-550 has been found in the plastid genomes of eukaryotic algae *Cyanophora paradoxa* (31), *Porphyra purpurea* (32) and a diatom *Odontella sinensis* (33) for which the complete plastid genome sequences have been determined. The psbV genes from all the three species of algae have a leader sequence in their N terminus, consistent with a thylakoid lumenal location of this extrinsic protein in plastid genomes from these three species of eukaryotic algae, however, the psbU gene for the 12-kDa protein was not found. Although the presence of the 12-kDa protein in *Cyanophora* and diatom has not been confirmed, this protein has been found in the red alga *C. caldarium* (26), and therefore, the psbU gene must be present in nuclear genome of the red alga. In fact, we have recently cloned the psbU gene from the red alga *C. caldarium*, which...
shows features of a typical nuclear gene coding for thylakoid luminal proteins, e.g., the presence of a bipartite transit peptide in its N terminus that is required for transport of the protein across both the envelope and thylakoid membranes of chloroplasts.3

The overall homology of the gene-derived amino acid sequence was 45.5–56.8% among three species of cyanobacteria and the red alga *C. caldarium* (26). This is significantly lower than those observed among different species of cyanobacteria and therefore suggests an evolutionary divergence in the oxygen-evolving complex between prokaryotic cyanobacteria and eu- karyotic red algae.

**Function of the 12-kDa Protein—Deletion of the psbU Gene** slightly decreased the PSI oxygen evolution. This effect was apparently caused by loss of the 12-kDa protein, because insertion of the kanamycin cassette downstream of the psbU gene had no detectable effects. This agrees with the previous results that the 12-kDa protein is required for cyanobacterial oxygen evolution. In addition, a significant loss of oxygen evolution accompanied by an apparent reduction in the growth rate (9, 11, 27, 34, 35). In contrast, the 12-kDa protein deletion mutant grew photoautotrophically at a rate very similar to that of the wild type strain.

The growth rate of the ΔpsbU mutant, however, was apparently lower than that of the wild type strain in the absence of Ca2+ or Cl−. This suggests a role of the 12-kDa protein in maintaining the optimum ion (Ca2+ and Cl−) environment required for cyanobacterial oxygen evolution. In addition, a shift to higher temperature in the ther- momoluminescence B- and Q-bands, as well as an increase in the intensity of the Q-band, were observed upon deletion of the psbU gene. These results may suggest a modification of the S-state, in particular the S2-state, upon depletion of the 12-kDa protein, which thus suggests a role of the 12-kDa protein in maintaining a functional structure of the algal PSI complex.

The double deletion mutant, ΔpsbUΔpsbV, showed a similar phenotype to that of the single deletion mutant ΔpsbV in terms of its oxygen evolution, growth in the presence or the absence of Ca2+ or Cl−, and thermoluminescence properties.2 This indicates that deletion of the psbU gene from the ΔpsbV mutant had essentially no further effect on the PSII oxygen-evolving complex, thus suggesting that the 12-kDa protein did not function in the single psbV deletion mutant lacking cyt. c-550. This agrees with the previous results that the 12-kDa protein cannot bind to and function in isolated PSI in the absence of either cyt. c-550 or the 33-kDa protein.

### REFERENCES

1. Bricker, T. M., and Ghonatikas, D. F. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yecm, C. F., eds) pp. 113–136, Kluwer Academic Publishers, Dordrecht, The Netherlands
2. Seidler, A. (1996) *Biochim. Biophys. Acta* 1277, 35–60
3. Nishiyama, Y. (1995) GenBank/EMBL/DDBJ data bases, accession number X93569
4. Shen, J.-R. (1996) *Biochim. Biophys. Acta* 1277, 35–60
5. Oto, T., and Inoue, Y. (1984) *FEBS Lett.* 166, 381–384
6. Miyao, M., and Murata, N. (1984) *FEBS Lett.* 170, 350–354
7. Kowahara, T., Miyao, M., Murata, T., and Murata, N. (1985) *Biochim. Biophys. Acta* 806, 283–289
8. Mayfield, S. P., Bennoun, P., and Boizieux, J.-D. (1987) *EMBO J.* 6, 313–318
9. Burnap, R. L., and Sherman, L. A. (1991) *Biochemistry* 30, 440–446
10. Shen, J.-R., and Inoue, Y. (1995) *Biochemistry* 32, 1825–1832
11. Shen, J.-R., Vermaas, W., and Inoue, Y. (1995) *J. Biol. Chem.* 270, 6901–6907
12. Shen, J.-R., Burnap, R. L., and Inoue, Y. (1995) *Biochemistry* 34, 12661–12668
13. Stewart, A. C., Ljungberg, Ulf., Åkerlund, H.-E., and Andersson, B. (1985) *Biochim. Biophys. Acta* 808, 353–362
14. Stewart, A. C., Sieczkowski, M., and Ljungberg, Ulf. (1985) *FEBS Lett.* 193, 175–179
15. Rolfe, S. A., and Bendall, D. S. (1989) *Biochim. Biophys. Acta* 973, 220–226
16. Wallace, T. P., Stewart, A. C., Pappin, D., and Howe, C. J. (1989) *Mol. Gen. Genet.* 216, 334–339
17. Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1992) *FEBS Lett.* 301, 145–149
18. Han, K.-C., Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1994) *FEBS Lett.* 355, 121–124
19. Yu, J., and Vermaas, W. F. J. (1990) *Plant Cell* 2, 315–322
20. Shen, J.-R., and Inoue, Y. (1995) *J. Biol. Chem.* 268, 20408–20413
21. Williams, J. G. K. (1988) *Methods Enzymol.* 167, 766–778
22. Thomas, P. E., Ryan, D., and Levin, W. (1976) *Anal. Biochem.* 75, 168–176
23. Oto, T., and Inoue, Y. (1986) *Biochim. Biophys. Acta* 850, 390–399
24. Kaneko, T., Sato, S., et al. (1996) *DNA Res.* 3, 109–136
25. von Heijne, G., Steppuhn, J., and Herrmann, R. G. (1989) *Eur. J. Biochem.* 180, 535–545
26. Enami, I., Murayama, H., Ohta, H., Kamo, M., Nakazato, K., and Shen, J.-R. (1995) *Biochim. Biophys. Acta* 1232, 208–216
27. Philbrick, J. B., Diner, B., and Zilinskas, B. A. (1991) *J. Biol. Chem.* 266, 13370–13376
28. Engels, D. H., Lott, L., Schmid, G. H., and Pistorius, E. K. (1994) *Photovyn.* Rev. 42, 227–244
29. Inoue, Y. (1996) *in* *Biophysical Techniques in Photosynthesis* (Anzem, J., and Hoff, A. J., eds) pp. 93–107, Kluwer Academic Publishers, Dordrecht, The Netherlands
30. Burnap, R. L., Shen, J.-R., Jursinic, P. A., Qian, M., Inoue, Y., and Sherman, L. A. (1992) *Biochemistry* 31, 7404–7410
31. Stirewalt, V. L., Michalowski, C. B., Hoff, A. J., and Swaisgood, A. J. (1995) *GenBank/EMBL/DDBJ data bases, accession number Z50501
32. Skotheim, J. A., and Munholland, J. (1996) *GenBank/EMBL/DDBJ data bases, accession number U38804
33. Kowalik, K. V., Stoebe, B., Schaffran, I., Koth, P., and Freier, U. (1996) *GenBank/EMBL/DDBJ data bases, accession number Z67773
34. Bockel, R., Masepohl, B., and Pistorius, E. K. (1991) *FEBS Lett.* 294, 59–63
35. Mayes, S. R., Cook, K. M., Self, J. S., Zhang, Z., and Barber, J. (1991) *Biochim. Biophys. Acta* 1060, 1–12