J. Plant Biol. 40(2) : 125~131 (1997)

Studies on Structure and Function of Photosystem II in Oxygenic Photosynthesis: Stoichiometry of Cytochrome *b*-559 in *Synechocystis* sp. PCC 6803

Cha, Hyeon Cheol and Gun-Sik Tae*

Department of Biological Sciences, College of Natural Sciences, Dankook University, Cheonan, Chungnam 330-714, South Korea

Cytochrome *b*-559 is an integral protein of the photosystem II (PSII) reaction center from both plants and cyanobacteria. Cytochrome *b*-559 has the unique structure of a heme cross-linked α- and β-subunit heterodimer. The stoichiometry of cytochrome *b*-559 (one or two copies) per the PSII reaction center has been the subject of controversy and the molar ratio of the heme group to the special chlorophyll P-680 has a number of significant implications on our understanding of the functional role of cytochrome *b*-559, the mechanism of electron donation in PSII, and the stoichiometry of the other redox-active reaction center components. In order to determine the number of the cytochrome *b*-559 heme in the PSII reaction center of *Synechocystis* sp. PCC 6803, the α- and β-subunits are covalently linked by use of the molecular genetic techniques. The resulting *psb*EF fusion mutant was able to grow photoautotrophically, implying that the PSII complexes are assembled and functional in thylakoids. This result supports the fact that there are two set of cytochrome *b*-559 in the PSII reaction center of *Synechocystis* sp. PCC 6803.

Key words: cytochrome *b*-559, photosystem II, *Synechocystis* sp. PCC 6803, site-directed mutagenesis, stoichiometry

The photosystem II (PSII) reaction centers of higher plants and cyanobacteria consist of four major polypeptides D1, D2, and the α- and β-subunits of cytochrome *b*-559. Studies on the structure of cytochrome *b*-559 are of interest because (i) the topography of its α- and β-subunits in the thylakoid membranes is known (Tae *et al.*, 1988; Vallon *et al.*, 1989; Tae and Cramer, 1994) and (ii) although the electron transport function is enigmatic (Cramer and Whitmarsh, 1977; Cramer *et al.*, 1993), it has the unusual aspect that it is involved in a response to stress, particularly photoinhibition (Thompson and Brudvig, 1988; Ohad *et al.*, 1990; Nedbal *et al.*, 1991; Ono *et al.*, 1995).

Cytochrome *b*-559 has the unique structure of a heme cross-linked dimeric α- and β-subunits. The stoichiometry of the α- and β-subunits of cytochrome *b*-559, which are encoded by the *psb*E and *psb*F genes, respectively, has been determined to be 1 to 1 (Widger *et al.*, 1984) and the heme group is ligated via bis-histidine through the hydrophobic interactions (Babcock *et al.*, 1985). Because the α- and β-subunits were recovered in 1:1 stoichiometry in a single fraction, the unit for heme coordination would have to be an heterodimer (Fig. 1A) if there were only one cytochrome *b*-559 heme per the PSII reaction center (Miyazaki *et al.*, 1989; Buser *et al.*, 1992). Moreover, the single histidine residue in each subunits, located at the 5th position from the NH_2-terminus of 26- and 25-residue-long hydrophobic membrane spanning domains of the α- and β-subunits, respectively (Hermann *et al.*, 1984), and the *bis*-histidine coordination would require that the α- and β-subunits have a parallel orientation in the thylakoid membrane bilayer (Cramer *et al.*, 1986). However, an *in situ* stoichiometry of two high-potential cytochrome *b*-559 hemes per the PSII reaction center in thylakoids (Selak *et al.*, 1984), two hemes per the reaction center in *Synechocystis* sp. PCC 6803 (MacDonald *et al.*, 1994), and a radiation cross-section in reaction centers corresponding to a dimeric cytochro-

*Corresponding author: Fax +82-417-551-9229
© 1997 by Botanical Society of Korea, Seoul

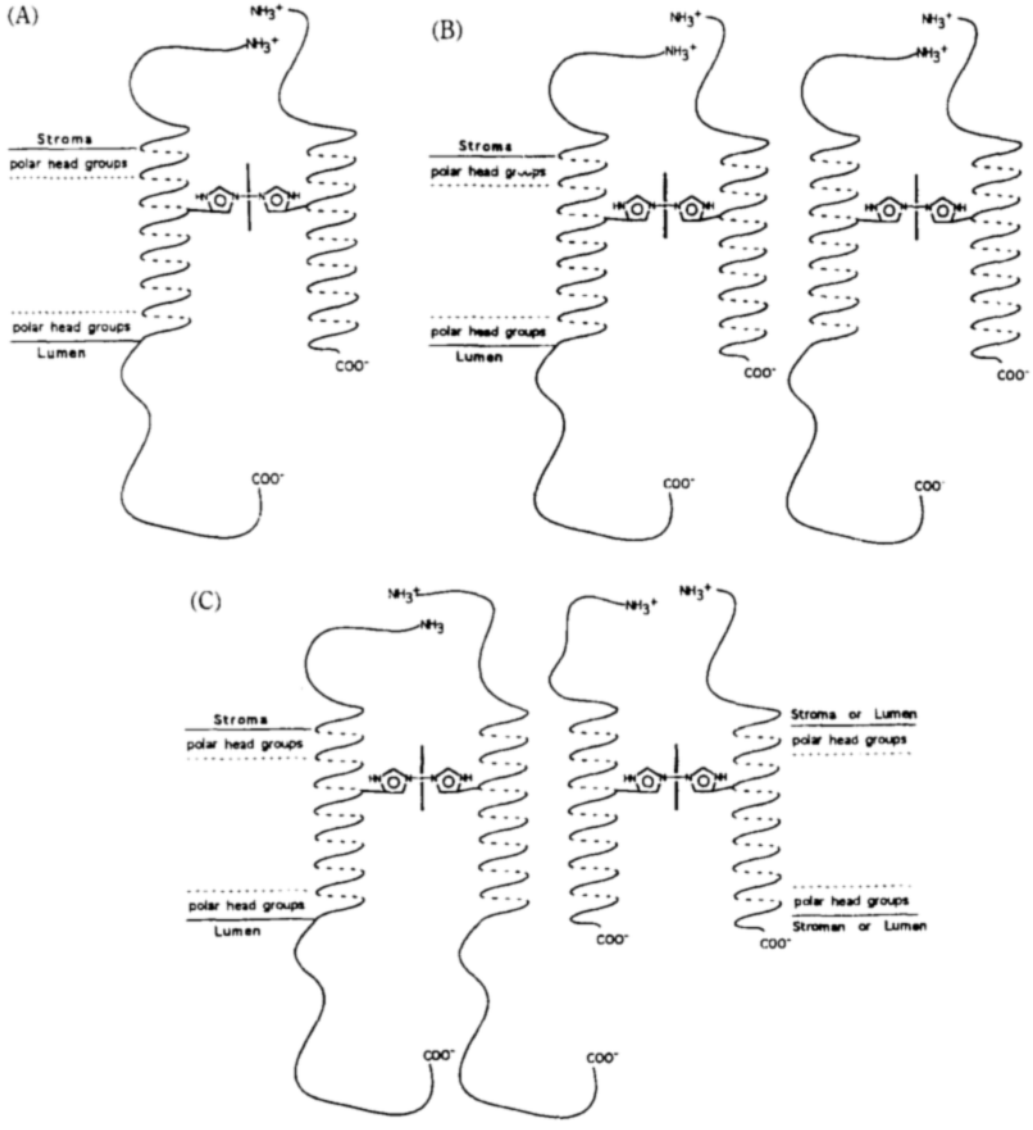

Fig. 1. Models for orientation of (A) the unit αβ heterodimer, (B) the diheme cytochrome *b*-559 αβ heterodimer, and (C) α_2 and β_2 homodimers in the thylakoid membrane bilayer. The heme iron (symbolized by a dot in the middle of the vertical bar) is coordinated by His-22 and His-17 of the α- and β-subunits, respectively. The NH_2- and COOH-termini of the α-subunit are positioned on the stromal and the lumenal sides of the membrane, respectively.

me *b*-559 (Takahashi and Asada, 1989) suggest that the cytochrome *b*-559 heme to the special chlorophyll (P-680) stoichiometry is two, but that one heme can be lost in more highly purified reaction center preparations. In the case of two hemes, the units for heme coordination and cytochrome *b*-559 structure could be $(\alpha\beta)_2$ heterodimers (Fig. 1B), as well as $(\alpha)_2$ and $(\beta)_2$ homodimers (Fig. 1C).

Elucidation of the number of heme(s) in the reaction center would help to solve the problem of the enigmatic function of cytochrome *b*-559. The molecular genetic techniques have been developed to introduce specific mutations in the PSII polypeptides of the photoheterotrophic cyanobacterium *Synechocystis* sp. PCC 6803 (Williams, 1988; Carpenter and Vermaas, 1989; Tae and Cramer, 1992) in order to analyze structure and function relationships of the PSII complex. In the present study the restriction enzyme sites (*Nhe*I) had been introduced by use of a site-directed mutagenesis technique at 3'-end region of the *psb*E gene and 5'-end region of the *psb*F gene. The DNA fragment between the mutation sites was removed by the restriction digestion and the ligation reactions. The *psb*E and *psb*F genes were covalently connected, resulting in the

*psb*EF fusion mutant, which is supposed to produce the α- and β-subunit-fused cytochrome *b*-559. On the basis of the capability of the photoautotrophic growth of the *psb*EF fusion mutant, the stoichiometry of cytochrome *b*-559 in the PSII reaction center of *Synechocystis* sp. PCC 6803 was discussed.

MATERIALS AND METHODS

Growth and manipulation of cyanobacterial strain

The wild-type and mutant strains of *Synechocystis* sp. PCC 6803 were grown either in liquid media (BG-11) or in agar plates under constant illumination [30-50 μEinstein m^{-2} sec^{-1} (Einstein=1 mole of photons) at 30°C (Rippka *et al.*, 1979; Williams, 1988). The 2-fold-concentrated BG-11 medium and 3% (w/v) Bacto-agar (Difco) were separately autoclaved and mixed, and then the supplements such as 5mM glucose, 50 μg/mL spectinomycin, and 30 μg/mL kanamycin were added when the solution was cooled down to 55-60°C. The *psb*EF deletion mutant (T1297; kindly donated from Dr. H. B. Pakrasi at Washington University) and the *psb*EF fusion mutant strains were propagated and maintained on plates containing 30 μg/mL kanamycin and 50 μg/mL spectinomycin, respectively. The growth rates of the wild-type and mutant strains were measured on the basis of the absorbance changes at 730 nm while growing in BG-11 media under constant illumination at 30°C in the presence and absence of 5 mM glucose. All the strains of *Synechocystis* sp. PCC 6803 were stored at -70°C in the presence of 20% glycerol.

Site-directed mutagenesis of the *psb*E and the *psb*F genes

In order to introduce the restriction enzyme *Nhe*I sites on both 3'-end region of the *psb*E gene and 5'-end region of the *psb*F gene of *Synechocystis* sp. PCC 6803, the synthetic oligonucleotides 5'-GAGTTTAATCAAGCTAGCATTTAATTGTTC-3' (Primer-1: G+C content: , 30%; complementary to the 3'-end region of the *psb*E gene) and 5'-TTTAGTTGGTAAGCTAGCATGCGTTTTGAC-3' (Primer-2: G+C content, 40%; complementary to 5'-end region of the *psb*F gene) were used as the site-directed mutagenesis primers (Zoller and Smith, 1983; Kunkel *et al.*, 1987). First, the mutation was introduced to the 3'-end region of the *psb*E gene. The phosphorylated primer-1 was annealed for 2 min at 70°C to the single-stranded DNA extracted from M13KO7 phage in a solution containing 50 mM NaCl, 20 mM $MgCl_2$, and 20 mM Tris-HCl, pH 7.5. The annealing mixture was cooled down to 30°C for 30 min. After the mutated primer-1 was annealed to the template, native T7 DNA polymerase, T4 DNA ligase, and 10-fold-concentrated synthesis buffer (5 mM each of dATP, dCTP, dGTP, and dTTP, 10 mM ATP, 50 mM $MgCl_2$, 20 mM DTT, and 100 mM Tris-HCl, pH 7.9) were added and incubated at 37°C for 30 min to synthesize the mutated complementary DNA strand. The resulting closed heteroduplex DNA was transformed directly into the *E. coli* MV1190 strain, in which dUTPase and uracil *N*-glycosylase were active. Several colonies were picked and plasmids were isolated from individual colonies. After the DNA sequences of the mutation site was confirmed by direct DNA sequencing of plasmids (Sanger *et al.*, 1977), the second mutation was introduced to 5'-end region of the *psb*F gene with the phosphorylated primer-2. All the procedures were repeated as mentioned before to get the double mutant in which the *Nhe*I restriction sites were introduced on 5'- end region of the *psb*F gene as well as 3'-end region of the *psb*F gene.

Construction of the recombinant *psb*EF fusion plasmid

The plasmid containing two *Nhe*I restriction sites on both 3'- and 5'- end regions of the *psb*E and *psb*F genes, respectively, was treated with *Nhe*I at 38°C for 1 hr. The digested products were separated on a 0.8% agarose gel and the band corresponding to the correct size was excised. After the extraction of DNA, the ligation reaction by use of T4 DNA polymerase was performed at room temperature for 1 hr and the ligation reaction mixture was transformed into *E. coli* MV1190 with the electrophorator (Bio-Rad). The *psb*EF fusion mutant was selected from the direct DNA sequencing of the plasmids extracted from individual colonies.

Transformation of the mutated *psb*EF fusion gene in pBluescript II SK$^+$ into the *psb*EF deletion mutant, T1297

A 1.5-mL sample of the culture grown to 2-5 × 10^8 cells/mL (A_{730}=0.25 corresponding to 10^8 cells/mL) was harvested by centrifugation at 4,500 g at room temperature for 10 min and resuspended in 200 μL of fresh BG-11 medium with 5 mM glucose. Two to five microliters of plasmid DNA (0.2 μg/μL) was added and incubated for 4 hrs in a sterile Falcon tube under the standard temperature and light conditions. The transformation mixture was incubated for another 18 hrs after adding 1 mL of BG-11 with 5 mM glucose and spreaded on Petri dishes containing

5 mM glucose and 50 μg/mL spectinomycin. Colonies of transformed cells could be seen in 2-3 weeks.

Isolation of the chromosomal DNA from *Synechocystis* sp. PCC 6803

One hundred milliliters of a late log-phase cell culture grown at 30°C was harvested by centrifugation at 5,000 g for 10 min, washed once with Tris-EDTA buffer (5 mM EDTA and 50 mM Tris-HCl, pH 7.5) and resuspended in saturated NaI solution (5 mL). Cells were heated to 65°C for 20 min and centrifuged at 10,000 g for 10 min. The sediment was washed once with Tris-EDTA buffer and resuspended in 10 mL of Tris-EDTA containing lysozyme (4 mg/mL). After incubation (37°C, 45 min), 2% (w/v) SDS, 2% (w/v) *N*-laurylsarcosine, and 100 μg/mL proteinase K (Boehringer) were added and incubated at 50°C for 1 hr. The lysate was extracted twice with a 1:1 mixture of phenol and chloroform (1 hr) and once with chloroform (1 hr) on the nutator. The upper phase was precipitated with 0.1 volume of 7.5 M ammonium acetate, pH 7.8, and 2 volumes of 100% ethanol. The chromosomal DNA dried under vacuum was redissolved in 2 mL of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and 40 μL of 1 M $MgCl_2$.

Identification of the mutation sites by Polymerase Chain Reaction of *Synechocystis* sp. PCC 6803

The *psb*EF fusion gene was amplified from the cyanobacterial genome by PCR with Taq DNA polymerase in order to ascertain that the correct mutations had been introduced into a cyanobacterium. For PCR of the *psb*EF fusion mutant, two synthetic oligonucleotides were used as primers for DNA polymerization: (i) 5'-CCCATATGTCAGGGACTACCG-GCGAGCGTC-3' (forward primer) to hybridize the complementary strand to the upstream region of the *psb*E gene including the start codon; (ii) 5'-GAAG-CTTCCCAACACAGCCACCAACAATAG-3' (reverse primer), complementary to the region in the middle of the coding strand of the *psb*L gene, which is located immediately downstream of the *psb*F gene. Twenty-five PCR cycles (each 60 sec at 94°C for denaturation of the double-stranded DNA followed by 60 sec at 55°C and 90 sec at 72°C for the hybridization and chain elongation, respectively) in a reaction medium (Promega, M1862) including 0.8 mM dNTP, 1 μM forward primer, 1 μM reverse primer, 0.5 μg of the chromosomal DNA, and 2 units of Taq DNA polymerase were sufficient to generate several micrograms of the amplified 0.5 kb of DNA fragment between hybridization sites of the oligonucleotides.

RESULTS AND DISCUSSION

The oxygen-evolving PSII complexes prepared from *Synechocystis* sp. PCC 6803, whose antenna size is approximately 46 chlorophylls, has been reported to contain 1.5-2.1 cytochrome *b*-559 per the PSII reaction center (MacDonald *et al.*, 1994). A review of the cytochrome *b*-559 literature suggests that there are two copies of the cytochrome *b*-559 heme in the PSII reaction center, but one of two may be lost easily at some purification step of the D1/D2/cytochrome

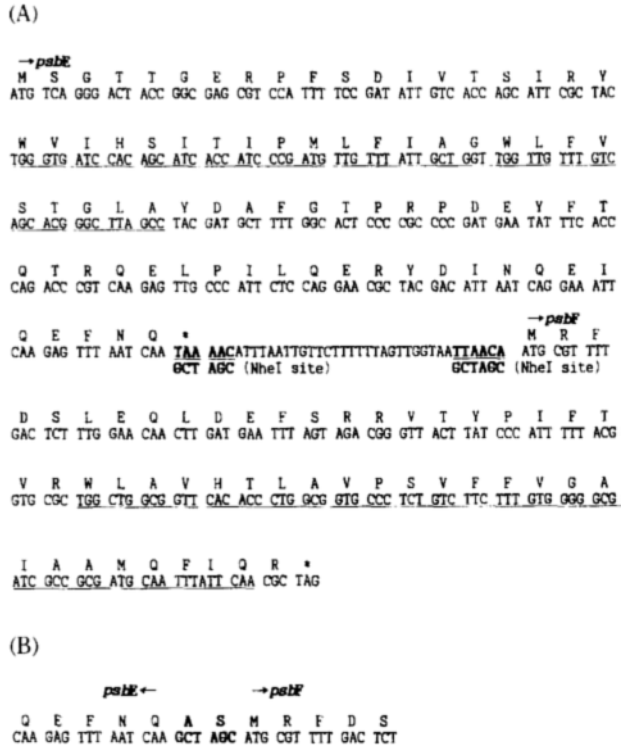

Fig. 2. Nucleotide and amino acid sequences of the cytochrome *b*-559 α- and β-subunits, encoded by the *psb*E and *psb*F genes in *Synechocystis* sp. PCC 6803. The amino acid sequence is written in one-letter code. The central hydrophobic domains of 24 and 25 amino acid residues of α- and β-subunits are underlined. The single His residues of each subunits are boxed. (A) The 3'- and 5'-end DNA sequences of the *psb*E (5'-TAAAA-3') and *psb*F (5'-TTAA-CA-3') genes, written in bold letter, are mutated to 5'-GCTAGC-3' by site-directed mutagenesis. (B) After the digestion of *Nhe*I, the new DNA sequence is inserted between the *psb*E and *psb*F genes, which encodes the amino acid Ala and Ser. The *psb*EF fusion protein has the full amino acid sequence of both α- and β-cytochrome *b*-559 in addition to Ala and Ser.

b-559 complex. However, a more detailed study of the data and the referenced literature reveals a number of inconsistencies and ambiguities, which are coming from the spectral overlaps in the red-visible region of cytochrome *f* (α-band at 554 nm), cytochrome *b*-559 (α-band at 559 nm), and cytochrome b_6 (α-band at 563 nm) and the significant variances in the number of chlorophyll per the PSII reaction center, purified from different methods. Therefore, there has been a difficulty to determine the stoichiometry of cytochrome *b*-559 in the PSII reaction center.

In order to examine the stoichiometry of the cytochrome *b*-559 heme in thylakoids of *Synechocystis* sp. PCC 6803, a mutant of which the *psb*E and *psb*F genes are covalently ligated was generated by introducing the restriction sites of *Nhe*I on both 3'-end region of the *psb*E gene and 5'-end region of the *psb*F gene (Fig. 2 A). The 3'-end DNA sequence of the *psb*E gene, 5'-TAAAA-3' including the stop codon (TAA), was site-directed mutagenized to 5'-GCTAG-3' by use of the mutated oligonucleotide primer 5'-GAGTTTAATCAAGCTAGCATTTAATTGTTC-3'. The 5'-end region of the *psb*F gene, 5'-TTAACA-3', was also mutagenized to 5'-GCTAGC-3' using the mutated primer, 5'-TTTAGTTGGTAAGCTAGCATG-CGTTTTGAC-3'. After the restriction digestion and ligation, the linking DNA fragment between the *psb*E and *psb*F genes was deleted and the new sequences (5'-GCTACG-3'; encoding the amino acid Ala and Ser) were introduced between the last amino acid codon of *psb*E (Gln) and the starting codon of *psb*F (Met) (Fig. 2 B). Therefore, the total number of amino acids of the cytochrome *b*-559 fusion protein will be 132 residues, in which the two hydrophobic membrane spanning domains could be located (i) from αY19 to αY44 and (ii) from βW25 to β49Q, originated from the α- and β-subunits, respectively. The *psb*EF fusion gene as well as the spectinomycin resistant gene ($Spec^R$) in the pBluescript SK II$^+$ vector was transformed into the *psb*EF deletion mutant strain of *Synechocystis* sp. PCC 6803 (Pakrasi *et al.*, 1988) to remove the background activity of cytochrome *b*-559. The mutated *psb*EF fusion gene is believed to be incorporated into the chromosomal DNA of T1297 through homologous recombination, resulting in the replacement of the kanamycin resistance gene (Km^R) in T1297 with the $Spec^R$ gene (Fig. 3). The presence of the nucleotide sequences encoding the additional amino acids, Ala and Ser, as well as the absence of the stop codon of the *psb*E gene, was confirmed by the direct PCR sequencing of the chromosomal DNA of the *psb*EF fusion mutant of *Synechocystis* sp. PCC 6803 (Fig. 4).

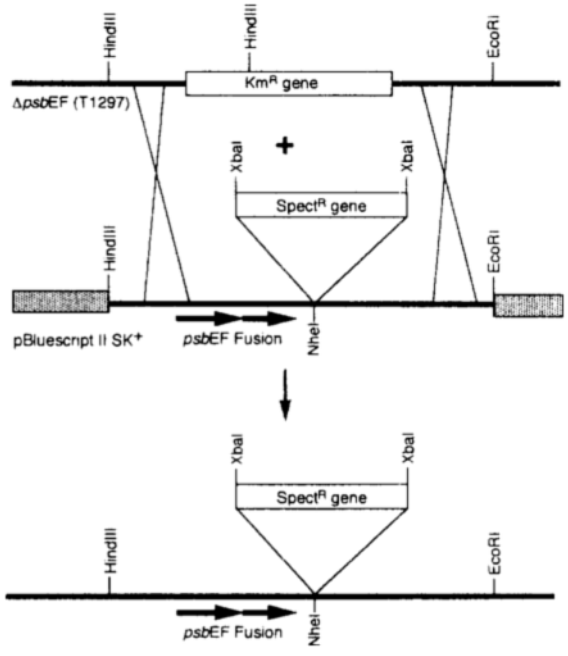

Fig. 3. Construction of the *psb*EF fusion mutant of *Synechocystis* sp. PCC 6803. The 2.2 kb *Hin*dIII/*Eco*RI fragment carrying the *psb*E and *psb*F genes encoding the cytochrome b559 α- and β-subunits is cloned into the pBluescript II SK$^+$ vector with restriction sites. The 2.0 kb *Xba*I fragment of the spectinomycin resistance gene from pRL 463 is ligated through the *Nhe*I site located downstream of the *psb*E gene. The 3'-end and 5'-end regions of the *psb*E and *psb*F genes were site-directed mutagenized to introduce the *Nhe*I sites. The kanamycin resistance gene of the *psb*EF deletion strain (T1297) is replaced by the spectinomycin resistance gene when homologous recombination takes place.

The *psb*EF fusion mutant was screened by the ability to grow on plates with spectinomycin. Since the copy number of the chromosomal DNA of *Synechocystis* sp. PCC 6803 was reported to be 6 to 8 (Williams, 1988), it took several restreaks to get the *psb*EF fusion mutant strain to a homozygote. The photoautotrophic competence and the growth rates were measured in the presence and absence of 5 mM glucose. The growth rate of the mutant was similar to that observed for the wild type under conditions in which the photosystem II activity is not required, in other words, in the presence of 5 mM glucose. Under photoautotrophic growth conditions, the *psb*EF fusion mutant was able to grow in the absence of the external carbon source even if the growth rate was decreased (Fig. 5).

Deletion of the *psb*EF genes (Pakrasi *et al.*, 1988),

Fig. 4. Identification of the mutation site by the chromosomal DNA sequencing of the *psb*EF fusion mutant of *Synechocystis* sp. PCC 6803. The *Nhe*I restriction enzyme site, 5'-GCTAGC-3', is flanked by the last codon of the *psb*E gene (CAA) and the start codon of the *psb*F gene (ATG).

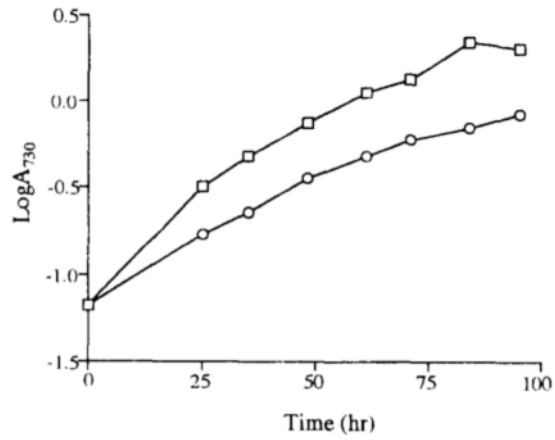

Fig. 5. Photoautotrophic growth curves of *Synechocystis* sp. PCC 6803 wild type and *psb*EF fusion mutant. Cells of wild type (□) and *psb*EF fusion mutant (○) were grown in BG-11 medium without glucose under constant illumination. The growth rates were monitored at 730 nm.

encoding the α- and β-subunits of cytochrome *b*-559 in *Synechocystis* sp. PCC 6803, resulted in loss of (i) assembly of the PSII reaction center complex and (ii) capability of the photoautotrophic growth. The PSII reaction center polypeptides, especially D1, are not detected in thylakoid membranes. These results imply that cytochrome *b*-559 has at least a structural role or an anchoring function in assembly of the PSII reaction center. Therefore, the photoautotrophic competence of the *psb*EF fusion mutant implies that the PSII reaction center is assembled properly in thylakoids and cytochrome *b*-559 is active in oxygenic photosynthesis. Since each of histidine residues are located at the 5th residue from the hydrophobic membrane spanning domains of both the α- and β-subunits, it may not be possible for each subunits to assemble through the heme group in the *psb*EF fusion mutant if there is only a set of cytochrome b559 in *Synechocystis* sp. PCC 6803. In other words, even if two hydrophobic domains, originated from the α- and β-subunits, have a chance to be inserted into the thylakoid membranes, the locations of the histidine residues are not likely to be aligned properly to ligate the heme group in thylakoids, because the histidine residues of α- and β-subunits would be located close to the cytoplasmic and lumenal sides of the thylakoid membrane, respectively. Therefore, the photoautotrophic competence of the *psb*EF fusion mutant supports the result that there are two set of cytochrome *b*-559 in PSII reaction center of *Synechocystis* sp. PCC 6803 (MacDonald *et al.*, 1994).

There might be two possibilities of how cytochrome *b*-559 of the *psb*EF fusion mutant is incorporated and assembled with other PSII components in thylakoids. Since the mutated cytochrome *b*-559 has two hydrophobic membrane spanning domains (αY19 to αY44 and βW25 to β49Q), originated from the α- and β-subunits, respectively, one possibility is that the membrane spanning domains of both the α- and β-subunits are inserted properly into the membrane, resulting in two heme groups located in one in cytoplasmic and the other in lumenal sides of the thylakoid membranes. The other possibility is that the hydrophobic domains only from α-subunits are embedded in the thylakoi membranes, which may incorporate only one heme group close to the cytoplasmic side, because the amino terminus of β-subunit is determined to be oriented to the cytoplasmic side of the thylakoid membrane (Tae *et al.*, 1988). Since two different kinds of antibodies are now available, which recognize the COOH-termial domain of α-subunit and the NH_2-terminal domain of β-subunit, it may not be impossible to determine the topography of the cytocrome *b*-559 fusion protein in the thylakoid membranes of *Synechocystis* sp. PCC 6803. Further investigations would be necessary to

understand the structural and functional relationships of cytochrome *b*-559 in the PSII reaction center.

ACKNOWLEDGEMENTS

This work was supported by a research grant from the Korean Ministry of Education (1995-1996) and a grant from Dankook University (1996).

LITERATURE CITED

Babcock, G.T., W.R. Widger, W.A. Cramer, W.A. Oertling, and J.G. Metz. 1985. Axial ligands of chloroplast cytochrome *b*-559: identification and requirement for a heme cross linked polypeptide structure. *Biochemistry* **24**: 3638-3645.

Buser, C.A., B.A. Diner, and G.W. Brudvig. 1992. Reevaluation of the stoichiometry of cytochrome *b*-559 in photosystem II and thylakoid membranes. *Biochemistry* **31**: 11441-11448.

Carpenter, S.D., and W.F.J. Vermaas. 1989. Directed mutagenesis to probe the structure and function of photosystem II. *Physiol. Plantarum* **77**: 436-443.

Cramer, W.A., and J. Whitmarsh. 1977. Photosynthetic cytochromes. *Ann. Rev. Plant Physiol.* **28**: 133-172.

Cramer, W.A., S.M. Theg, and W.R. Widger. 1986. On the structure and function of cytochrome *b*-559. *Photosyn. Res.* **10**: 393-403.

Cramer, W.A., G.-S. Tae, P.N. Furbacher, and M. Bottger. 1993. The enigmatic cytochrome *b*-559 of oxygenic photosynthesis. *Physiol. Plantarum* **88**: 705-711.

Hermann, R.G., J. Alt, B. Schiller, W.R. Widger, and W.A. Cramer. 1984. Nucleotide sequence of the gene for apocytochrome *b*-559 on the spinach plastid chromosome. *FEBS Lett.* **176**: 239-244.

Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Method Enzymol.* **146**: 367-382.

MacDonald, G.M., R.J. Boerner, R.M. Everly, W.A. Cramer, R.J. Debus, and B.A. Barry. 1994. Comparison of cytochrome *b*-559 content in photosystem II complexes from spinach and *Synechocystis* sp. PCC 6803. *Biochemistry* **33**: 4393-4400.

Miyazaki, A., T. Shina, Y. Toyoshima, K. Gounaris, and J. Barber. 1989. Stoichiometry of cytochrome *b*-559 in photosystem II. *Biochim. Biophys. Acta* **975**: 142-147.

Nedbal, L., C. Gibas, and J. Whitmarsh. 1991. Light saturation curves show competence of the water splitting complex in inactive photosystem II reaction centers. *Photosyn. Res.* **30**: 85-94.

Ohad, I., N. Adir, H. Koike, D.J. Kyle, and Y. Inoue. 1990. Mechanism of photoinhibition *in vivo*. *J. Biol. Chem.* **265**: 1972-1979.

Ono, T., T. Noguchi, and Y. Nakajima. 1995. Characteristic changes of function and structure of photosystem II during strong-light photoinhibition under aerobic conditions. *Biochim. Biophys. Acta* **1229**: 239-248.

Pakrasi, H.B., J.G.K. Williams, and C.J. Arntzen. 1988. Targeted mutagenesis of the *psb*E and *psb*F genes blocks photosynthetic electron transport: evidence for a functional role of cytochrome *b*-559 in PSII. *EMBO J.* **7**: 325-332.

Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman, and R.T. Stainer. 1979. Genetic assignment, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**: 1-61.

Sanger, F., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.

Selak, M.A., B.E. Koch-Whitmarsh, and J. Whitmarsh. 1984. Evidence for a heterogeneous population of high potential cytochrome *b*-559 in the thylakoid membrane. In *Adv. in Photosyn. Res.* (Sybesma, C. ed.), vol. I: pp. 493-496. Martinus Nijhoff Publishers, The Hague/Boston/Lancaster.

Tae, G.-S., M.T. Black, W.A. Cramer, O. Vallon, and L. Bogorad. 1988. Thylakoid membrane protein topography: Transmembrane orientation of the chloroplast cytochrome *b*-559 *psb*E gene product. *Biochemistry* **27**: 9075-9080.

Tae, G.-S., and W.A. Cramer. 1992. Truncation of the COOH-terminal domain of the *psb*E gene product in *Synechocystis* sp. PCC 6803: Requirements for photosystem II assembly and function. *Biochemistry* **31**: 4066-4074.

Tae, G.-S., and W.A. Cramer. 1994. Topography of the heme prosthetic group of cytochrome *b*-559 in the photosystem II reaction center. *Biochemistry* **33**: 10060-10068.

Takahashi, M., and K. Asada. 1989. Molecular structure of a high potential form of thylakoid cytochrome *b*-559 as determined by radiation inactivation. *Plant Cell Physiol.* **30**: 915-921.

Thompson, L.K., and G.W. Brudvig. 1988. Electron donation in photosystem II. *Isarel J. Chemistry* **28**: 121-128.

Vallon, O., G.-S. Tae, W.A. Cramer, D. Simpson, G. Hoyer-Hansen, and L. Bogorad. 1989. Visualization of antibody binding to the photosynthetic membrane: the transmembrane orientaion of cytochrome *b*-559. *Biochim. Biophys. Acta* **975**: 132-141.

Widger, W.R., W.A. Cramer, M. Hermodson, D. Meyer, and M. Gullifor. 1984. Purification and partial amino acid sequence of the chloroplast cytochrome *b*-559. *J. Biol. Chem.* **259**: 3870-3876.

Williams, J.G.K. 1988. Construction of specific mutations in the photosystem II photosynthetic reaction center by genetic engineering methods in the cyanobacterium Synechocystis 6803. *Method Enzymol.* **167**: 766-778.

Zoller, M.J., and M. Smith. 1983. Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors. *Method Enzymol.* **100**: 468-500.

Received May 12, 1997
Accepted July 17, 1997