S8-033

A novel slr1050 protein is involved in the function of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803

EH Kim[1], EK Song[1], Y-H Chung[2], YM Park[2] and C-H Lee[1]

[1]*Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea. chlee@hyowon.cc.pusan.ac.kr*

[2]*Biomolecule Research Team, Korea Basic Science Institute, 52 Yeoeun-dong, Yusung-ku, Taejon, 305-333, Korea.*

Keywords: Photoinhibition, Photosystem II, Synechocystis sp. PCC 6803, Cyanobacterium, Transposon

Introduction

When photosynthetic organisms are exposed to an irradiance greater than can be used in photosynthesis, they are liable to be damaged in a process called photoinhibition. In recent years, a large number of studies have shown that the primary site of damage in high light is the photosystem (PS) II reaction center. A number of different mechanisms for this damage have been identified, that invoke damage to the donor side or the acceptor side of the reaction center (Powles, 1984).

To facilitate the genetic analysis of the selected mutants, we employed the random transposon mutagenesis technique, used for *Synechococcus* sp. PCC 7942 (Andersson et al, 2000), for *Synechocystis* sp. PCC 6803 (*Synechocystis* 6803) to generate a large number of mutants for the selection of mutants of related to the sensitivity to high light stress. A novel gene responsible for the phenotype was identified as slr1050, and the biochemical characteristics of the slr1050::Tn5 mutant were investigated in relation to the function of the impaired gene.

2. Materials and methods

2.1. Strains and culture conditions

A glucose-tolerant wild type (WT) strain of *Synechocystis* 6803 was grown in BG 11 medium supplemented with 5 mM (HEPES)-NaOH (pH 7.5) at 28^{o}C under continuous illumination at 20~25 μmol m^{-2} s^{-1}. Transposon tagging mutants were maintained on the 10μg/ml kanamycin added media.

2.2. Identification of transposon flanking sequence

Genomic DNA was completely digested by *Taq* I restriction enzyme. The digested DNA products were self-ligated using DNA ligase. To amplify transposon flanking sequence, inverse polymerase chain reaction (PCR).

2.3. Measurement of electron transport rate and Chl a fluorescence induction transients

The electron transport rates were measured at 25^{o}C and the 1600 μmol m^{-2} s^{-1} actinic light (projection lamp) using a Clark-type oxygen electrode. Chl (Chlorophyll) *a* fluorescence induction transients of intact cells were measured by a pulse-amplitude-modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany).

2.4. Photoinhibition

Cells were grown mixotrophically at 25 μmol m^{-2} s^{-1}. The concentration of cells were adjusted to Chl 10 μg/ml and then exposed to 200 μmol m^{-2} s^{-1} for 10 min.

3. Results and Discussion

3.1. Screening and genetic characterization of a high light sensitive mutant

Under photoautotrophic conditions at low light intensity (20 μmol m^{-2} s^{-1}), a strain grew normally as WT did. At medium light intensity (60 μmol m^{-2} s^{-1}), however, the growth rate of the mutant was lower than that of WT. At photoinhibitory high light intensities (100 and 200 μmol m^{-2} s^{-1}), the growth of mutant was severely retarded (data not shown). To determine the Tn*5* insertion site, we performed inverse PCR, and a 400 bp product was directly sequenced. The DNA sequence analysis by BLAST search in Cyanobase on the web (http://www.kazusa.or.jp/cyano) showed that Tn*5* was inserted at 118 bp 5' from the start codon of the slr1050 gene. Based on the nucleotide sequence, slr1050 is a polypeptide of 477 amino acids with a predicted molecular weight of 54.7 kDa, which has ten putative transmembrane spans according to DAS transmembrane prediction database (http://www.sbc.su.se/~miklos/DAS/).

3.2. Photosynthetic properties of the slr1050::Tn5 mutant

To examine the photosynthetic properties of the mutant, we first compared its photosynthetic electron transfer rate with the rate in WT. As shown in Table 1, the whole chain and PS II-mediated oxygen evolution rate in slr1050::Tn*5* mutant were 60~70% of the values in WT. However, the PS I activity in the slr1050::Tn*5* mutant was normal. To further investigate a possible lesion site in PS II, Chl *a* fluorescence transients were measured in the presence or absence of 3-(3,4-dichlorophenyl)-1,1-

dimethylurea (DCMU) (Fig. 1). A maximally fluorescent state results from the accumulation of the $P680^+Q_A^-$ state, (P680, primary electron donor; Q_A, primary plastoquinon electron acceptor). In the absence of DCMU, the fluorescence emitted from the mutant was lower than that of WT (Fig. 1A). The lesion site of PS II can be either in the donor or in the acceptor side of the reaction center. If the electron donation from the water oxidizing complex to PS II is damaged, a decreased rate of electron donation to oxidized $P680^+$ allows charge recombination between $P680^+$ and Q_A^- to effectively compete with the forward reaction (Erickson et al., 1989; Pablo et al., 2000). Lower fluorescence yield can be expected if the electron flow is faster after Q_A^- in the mutants. To check this possibility, fluorescence induction was measured in the presence of DCMU to block electron flow after Q_A^-. The relative fluorescence yield of the mutant compared with the yield of WT did not altered significantly in the presence of DCMU (Fig. 1B). This result implies that the electron transport flow on the donor side is partially impaired in the slr1050::Tn*5* mutant.

	Activities (μmol O_2 evolved $mgChl^{-1}h^{-1}$) WT	Activities (μmol O_2 evolved $mgChl^{-1}h^{-1}$) slr1050::Tn*5*
H_2O to HCO_3^- (whole chain)	300	188
H_2O to pBQ (PS II)	281	172
TMPD/Asc to MV (PS I)	-150	-155

Table 1. Photosynthetic electron transport activities of *Synechocystis* 6803 WT and slr1050:;Tn*5* mutant
Asc, sodium ascorbate; MV, methyl viologen; PBQ, *p*-benzoquinone; TMPD, 2,3,5,6-tetramethyl-*p*-phenelynediamine

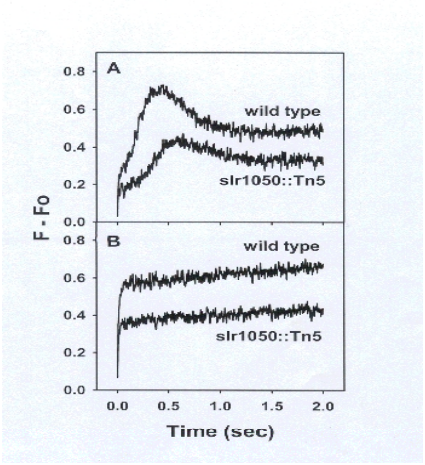

Fig.1. Chl *a* fluorescence induction transients in WT *Synechocystis* 6803 and slr1050::Tn5 mutant

3.3. Susceptibility of PS II to photoinhibition in the slr1050::Tn5 mutant

Due to photoinhibition at 200 μmol m^{-2} s^{-1} for 50 min, variable fluorescence (Fv/Fm) decreased 50% in the case of slr1050::Tn*5* mutant and 30% in the case of WT (Fig. 2). To examine the possible lesion sites of photoinhibition, electron transfer activity was measured (Table 2). After this photoinhibitory treatment, the whole and PS II photosynthetic electron transport activities of the slr1050::Tn*5* mutant was significantly

lower than those of WT. However, there was no significant differences in activity of PS I between the mutant and WT. This result suggests that the slr1050::Tn*5* mutant is a very photosensitive strain, and PS II seem to be labile to photoinhibition.

In conclusion, slr1050 protein is a membrane protein, but it might not be a structural protein of PS II because the most polypeptide components of cyanobacterial

	Activities (μmol O_2 evolved $mgChl^{-1}h^{-1}$) WT	Activities (μmol O_2 evolved $mgChl^{-1}h^{-1}$) slr1050::Tn*5*
H_2O to HCO_3^- (whole chain)	237	137
H_2O to pBQ (PS II)	237	95
TMPD/Asc to MV (PS I)	142	144

Table 2. Photosynthetic electron transport activites of photoinhibited *Synechocystis* 6803 WT and slr1050::Tn*5* mutant.

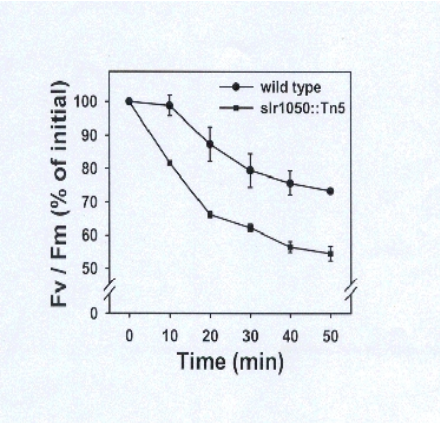

Fig. 2. Decrease in photochemical efficiency of WT *Synechocystis* 6803 (circle) and slr1050::Tn*5* mutant (square) exposed to 200 μmol m^{-2} s.

investigated. The mutation of the slr1050 gene causes a marked reduction in quantum yield and photosynthetic electron transport activity of PS II with no apparent significant effects on the function of PS I. However, its growth at low light (25 μmol m^{-2} s^{-1} or lower) was normal. Based on the impaired capacity of electron donation to PS II reaction centers as well as the apparent photoinhibition of PS II activities in the slr1050::Tn*5* mutant, we suggest that slr1050 protein is a membrane protein which facilitates and protects the electron donation to PS II. The subcellular location and the specific function of slr1050 in relation to the function of PS II are under investigation.

References

Andersson CR, Tsinoremas NF, Shelton J, Lebedeva NV, Yarrow J, Min H and Golden SS (2000) *Methods in Enzymolgy* 305, 527-542.

Erickson JM, Pfister K, Rahire M, Togasaki RK, Mets L and Rochaix JD (1989) *Plant Cell* 1, 361-371.

Pablo ES, Tolle J, Bader KP, Pistorius EK and Michel KP (2000) *Photosynthesis Research* 63, 145-157.

Powles SB (1984) *Annual Reviews of Plant Physiology and Plant Molecular Biology* 35,15-44.