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Functional analysis of PSII-T protein of photosystem II complex from the thermophilic cyanobacterium, *Thermosynechococcus* (formerly *Synechococcus*) *elongatus* BP-1

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Introduction

PSII is a multi-subunit pigment-protein complex with the enzymatic activity of light-dependent water-oxidizing plastoquinone reductase. The PSII complex has been shown to be in a dimeric form in thylakoid membranes of both cyanobacteria and plants, although its precise role has not yet been established (Nield et al. 2000). Possible involvment of PSII-W in dimerization was reported in the antisense mutant of Arabidopsis (Shi et al. 2000). However, this subunit is absent in the cyanobacterial genome, in spite of the dimeric form. In this report, we cloned and disrupted *psbT* in the thermophilic *Thermosynechococcus elongatus* BP-1 and isolated the active PSII complex. Results suggested that *psbT* is dispensable for the photoautotrophic growth but is necessary for dimerization of the complex.

Materials and methods

The thermophilic cyanobacterium, *Thermosynechococcus elongatus* BP-1 was derived from a hot spring in Beppu district in Japan (Yamaoka et al. 1978). For cloning of *psbB* and *psbT*, a two-step PCR method was employed according to the consensus amino acid sequence of CP47. *psbT* was insertionally disrupted with a promoter-modified chloramphenicol-resistant cassette (Katoh and Ikeuchi 2001). The resulting plasmid DNA was introduced into *T. elongatus* cells by electroporation and transformants were selected on a chloramphenicol-containing agar plate (Katoh and Ikeuchi 2001, Katoh et al. 2001). Complete segregation of the mutant genome as a result of double homologous recombination was confirmed by PCR.

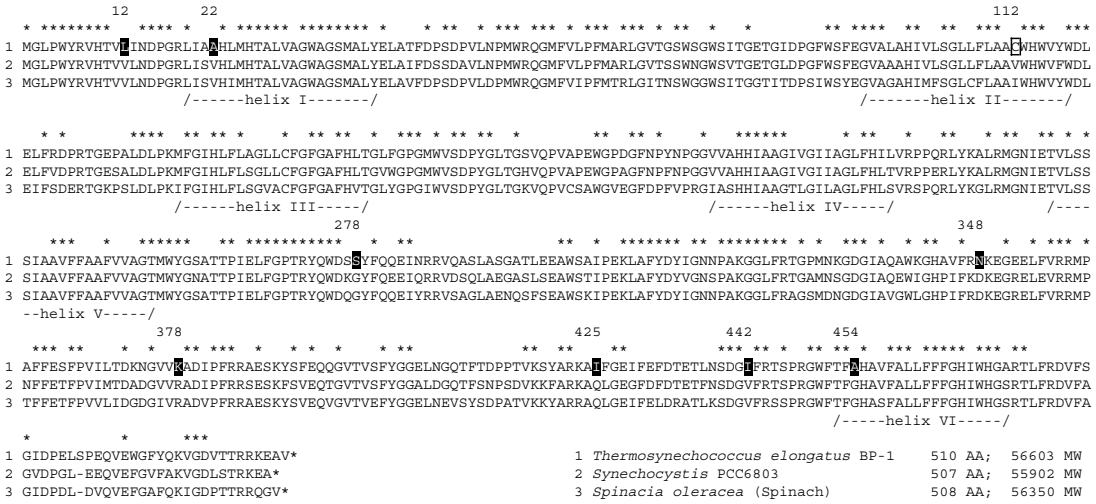

Fig. 1 Sequence alignment of CP47. Amino acid residues conserved between *T. elongatus* and 15 species including cyanobacteria and plants are indicated by asterisks. Amino acid residues, which were not conserved only in *T. elongatus* were indicated by black box. Cys present only in *T. elongatus* was indicated by white box. Line under the sequence indicates membrane-spanning region.

Preparation of thylakoids and isolation of the PSII core complex was performed according to (Katoh and Ikeuchi 2001). In essence, thylakoid membranes were solubilized with n-heptyl-β-D-thioglucoside and crude PSII particles were collected by differential centrifugation. Monomeric and dimeric forms of PSII complex were resolved by MonoQ column chromatography with the linear gradient from 100 mM to 350 mM NaCl. Size of the PSII complex was confirmed by gel filtration chromatography using Superdex-200 (PC3.2/30, SMART System, Amersham Pharmacia Biotech). SDS-urea-PAGE was performed according to Ikeuchi and Inoue (1988). Oxygen-evolving activity of thylakoids (final 30 µg Chl /ml) or PSII particles (final 4 µg Chl /ml) was measured at 25 °C using a Clark-type oxygen electrode with saturating light of about 3000 µE/m^2/s in the presence of 2,6DCBQ as an electron acceptor.

Thermosynechococcus elongatus	PSII-T	METITYVFIFACIIALFFFAIFFREPPRITKK*
Thermosynechococcus vulcanus	PSII-N	METITYVLIFA?IIALFFF/

Fig. 2 Sequence alignment of PSII-T and PSII-N. Amino acid sequence of PSII-T was deduced from the nucleotide sequence. Partial amino acid sequence under the name of PSII-N was obtained by N-terminal sequencing (Ikeuchi et al. 1989).

Results and discussion

Amino acid sequences of CP47 and PSII-T, which are deduced from *psbB* and *psbT* in *T. elongatus*, respectively, are highly homologous to those from other cyanobacteria and plants. Notably, CP47 of *T. elongatus* was unique at eight amino acid positions, which are conserved in the other organisms (Fig. 1). They include a position 348 at the lumenal side (Gleiter et al. 1995), where the conserved negative charge was lost in this organism. Further, CP47 and PSII-T of *T. elongatus* had additional Cys residues in the middle of membrane-spanning segments (Cys 112 of CP47 in Fig. 1

and Cys 12 of PSII-T in Fig. 2). This may suggest close interaction between the two proteins encoded by genes in an operon and might explain the thermostability of the PSII complex.

The *psbT*-disrupted mutant could grow photoautotrophically at a rate comparable to wild type. Consistently, there was no appreciable difference in the oxygen-evolving activities of cells, thylakoids, or PSII complexes between the mutant and wild type. This is in contrast with the lower activities of the other PSII mutants such as *psbX* or *psbV*-disruptants in *T. elongatus* (Katoh and Ikeuchi 2001, Katoh et al. 2001).

Polypeptide composition of the PSII core complexes was analyzed by SDS-urea-PAGE (Fig. 3). There is no apparent difference between the mutant and wild type except for the absence of a 4.7 kDa band in mutant. In our earlier work with *Thermosynechococcus vulcanus,* we demonstrated by N-terminal sequencing that the 4.7 kDa band consisted of two proteins, which correspond to two conserved ORFs designated *psbM* and *psbN* of various chloroplast genomes (Ikeuchi et al. 1989). However, sequence comparison revealed that one of the 4.7 kDa sequences is almost identical to the deduced sequence of *psbT* (Fig. 2). Thus, we revise our assignment of the 4.7 kDa PSII protein from PSII-N to PSII-T, deleting *psbN* from the list of PSII genes. The PSII core complexes were fractionated by MonoQ column chromatography (Fig. 4). The elution profile of wild type (panel A) and the mutant (panel B) showed two peaks at about 200 mM and 220 mM NaCl, respectively. Considering the gel filtration profiles, the oxygen-evolving activities, and the polypeptide composition of each peak fraction (data not shown), the peak 1 fraction of wild

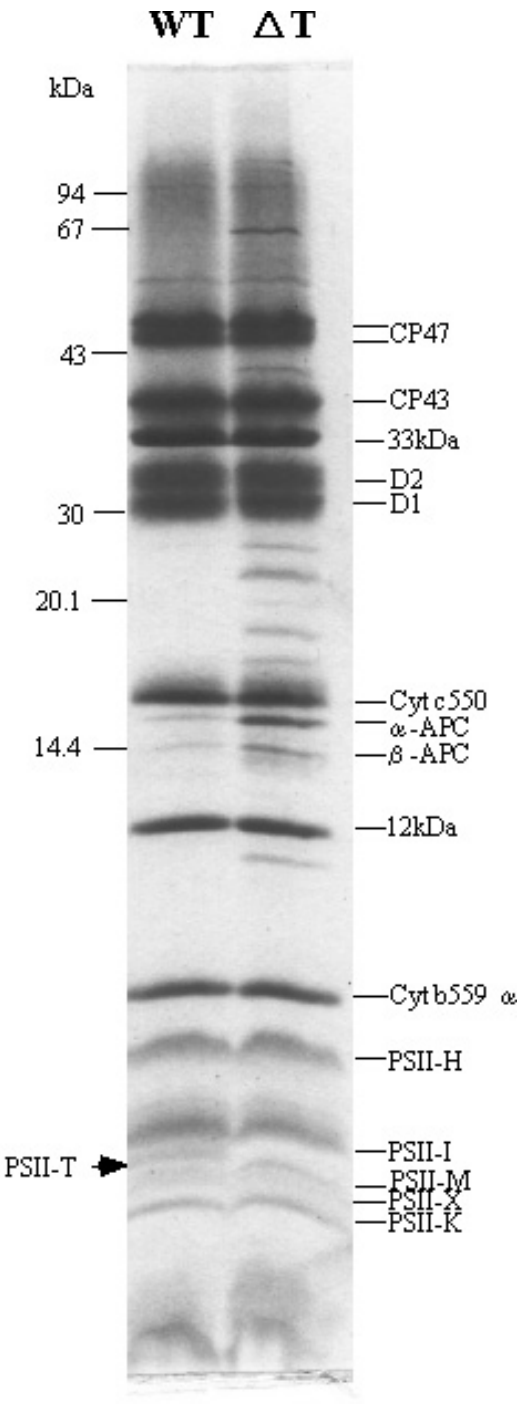

Fig. 3 SDS-urea-PAGE profile of PSII core complex from wild type (WT) and *psbT*-disrupted mutant (ΔT) of *T. elongatus.* The position of 4.7 kDa PSII-T protein is indicated with an arrow. Positions of molecular size markers are indicated on the left.

type and the peak 3 fraction of the mutant were the monomeric PSII, while the peak 2 fraction of wild type was the dimeric PSII. Although peak 4 of the mutant appears to correspond to the dimeric PSII, its peak height is small. In our experimental conditions, even wild-type PSII yielded both monomeric and dimeric forms in contrast to predominance of the dimer in other reports (Kuhl et al. 2000). Taking this into account, our data suggest that PSII-T protein is not essential but does contribute to the dimer formation of the PSII complex.

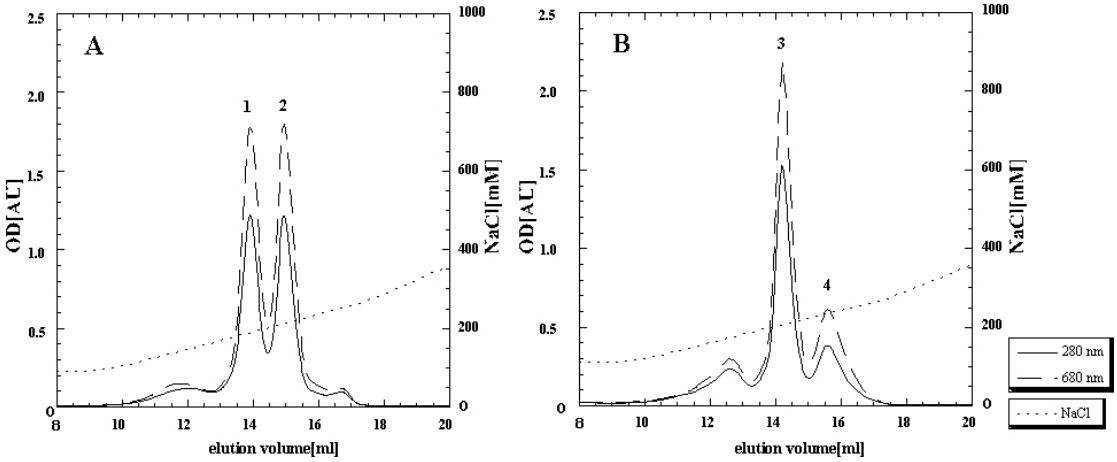

Fig. 4 Elution profile of PSII core complex from wild type (panel A) and *psbT*-disrupted mutant (panel B) of *T. elongatus* in MonoQ ion-exchange chromatography

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