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Chloroplast-encoded small polypeptide, PsbT, is required for the efficient recovery of photodamaged photosystem II

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Introduction

Light energy is essential for photosynthesis and is converted into redox energy by two photosystems, photosystem I (PSI) and photosystem II (PSII). However it is known that excess light causes damage to photosynthetic apparatus (Aro *et al.* 1993). It is generally accepted that one of the main targets of photoinhibition is PSII. The PSII forms chlorophyll-protein complex consisting of a number of cofactors and subunits. Improved SDS-polyacrylamide gel electrophoresis has revealed that the PSII complex contains more than ten hydrophobic polypeptides smaller than 10 kDa (Ikeuchi 1992). Some of them might be required for assembly, stability, and/or optimal function of PSII (Takahashi 1998), although functional role of these small polypeptides has not been fully elucidated.

The 4 kDa PsbT polypeptide encoded by the chloroplast *psbT* gene has a putative transmembrane helix and is associated with the PSII core complex. The *psbT* deletion mutants (Δ*psbT*) generated from the green alga *Chlamydomonas reinhardtii* are able to grow photoautotrophically like wild type in the moderate light and their activity and amount of PSII are reduced only slightly. Therefore PsbT is not essential for structure and function of PSII complex (Monod *et al.* 1994). However, the cell growth and PSII activity of Δ*psbT* were significantly impaired under light stress condition, suggesting that the PSII is more photosensitive in the absence of PsbT. Here, we have characterized the PSII activity during and after strong illumination and concluded that the PsbT is required for efficient repair of photodamaged PSII.

Materials and methods

C. reinhardtii wild-type strain 137c and a *psbT* deletion mutant (Δ*psbT*) were grown to mid-log phase (4 x 10^6 cells/ml) in Tris-acetate-phosphate (TAP) medium (Gorman *et al.* 1965) at 25 ^oC in the dim light (10 μmol photons/m^2/s). Effects of strong light

illumination on PSII activity were investigated by exposing cells (10 μg Chl/ml) to strong light (4,000 μmol photons/m^2/s) or incubating cells in the dark as a control. O_2 evolution of cells (10 μg Chl/ml) was measured with a Clark-type O_2 electrode using actinic light of intensity of 5,000 μmol photons/m^2/s in TAP medium containing 0.2 mM phenyl-*p*-benzoquinone.

Results

Figure 1 shows the effects of strong light on PSII activity. Strong light illumination resulted in rapid reduction of the O_2 evolution to approximately 35% of the original level in wild-type cells. After the initial rapid reduction, the activity subsequently restored partially to more than 50% of the original level after 4 h illumination. In Δ*psbT* cells, a very similar initial reduction of the activity was observed but to a larger extent. In addition, a subsequent increase of the activity was scarcely observed, so that the activity was decreased to less than 15% of the initial level after 12 h illumination. In the dark, no decrease was observed in both strains (data not shown). The wild-type cells contained constant amounts of major PSII proteins while the Δ*psbT* cells showed gradual reduction of the PSII proteins during the strong light illumination (data not shown).

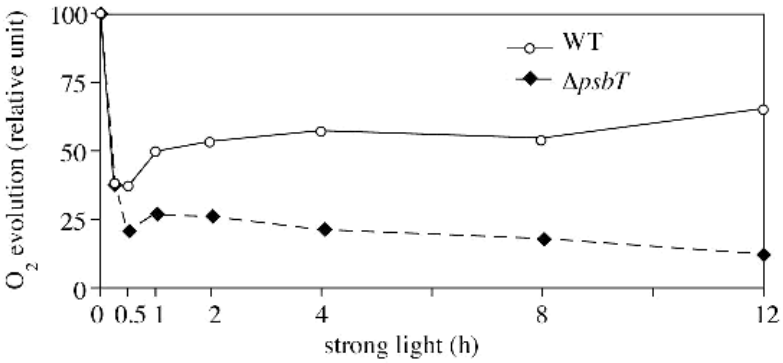

Figure 1 Effects of strong light illumination (4,000 μmol photons m^{-2} s^{-1}) on the PSII activity.

To investigate whether the enhanced photoinhibition in Δ*psbT* is due to an accelerated photoinactivation or an inefficient repair of the photodamaged PSII, the rate of photoinactivation of O_2 evolution was measured in the presence of chloramphenicol which blocks the repair. Figure 2 shows that the activity of wild type and Δ*psbT* decreased at the same rate with a half-decay time of 12 min. The photodegradation of major PSII proteins occurred at the same rate in both strains (data not shown). These results indicate that PsbT is not involved in the protection of PSII against strong light but could be required for the efficient repair of photodamaged PSII.

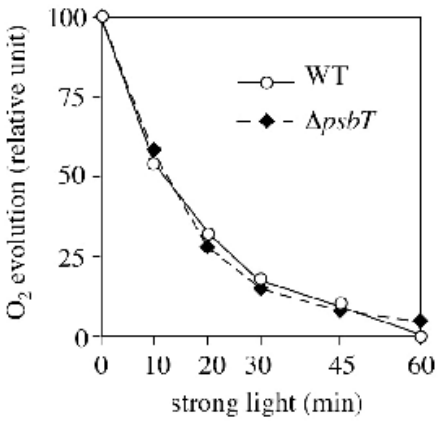

Figure 2 Light-induced inactivation of O_2-evolution in wild-type and Δ*psbT* cells. The cells were illuminated at 4,000 μmol photons m^{-2} s^{-1} in the presence of chloramphenicol (200 μg ml^{-1}).

To confirm the involvement of PsbT in the repair process, we analyzed the recovery of PSII activity after photoinhibition. Figure 3 shows that the photodamaged activity of O_2 evolution was recovered to the initial level after 60 min of the low light illumination in wild-type cells while the recovery was approximately four times slower in Δ*psbT* cells. These observations clearly indicate that PsbT is required for the efficient recovery of photodamaged PSII.

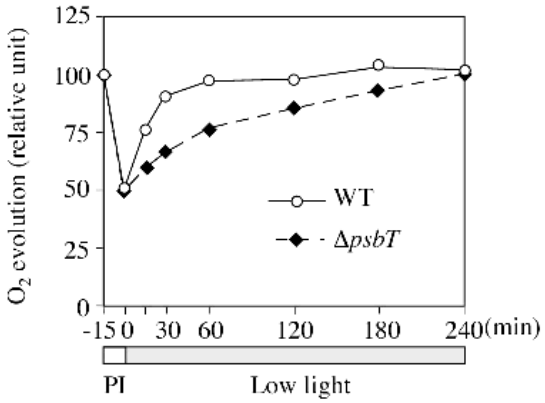

Figure 3 Recovery of the O_2 evolution after photoinhibition (PI). Wild-type and Δ*psbT* cells were photoinhibited by the strong light (4,000 μmol photons m^{-2} s^{-1}) for 15 min. Recovery of the activity was followed in the low light (40 μmol photons m^{-2} s^{-1}) .

Discussion

In the present study, we have shown that strong light illumination results in significantly reduced steady-state levels of PSII activity in the Δ*psbT* mutant cells compared with wild-type cells. The absence of PsbT did not affect the photoinactivation rate of PSII, while recovery of photoinhibited PSII activity was remarkably delayed. Thus PsbT is required for efficient recovery of the photodamaged PSII complex. The inefficient recovery of the photodamaged PSII leads to the reduced steady-state level of PSII activity and probably the defect in cell growth in strong light. Thus this is the first evidence that one of the small hydrophobic polypeptides of PSII is involved in the repair process of the photodamaged PSII.

The repair of the photodamaged PSII activity is a multi-step process (Aro *et al.* 1993). This includes replacement of photodamaged D1 and other PSII proteins by newly

synthesized copies, and integration and reactivation of cofactors that were lost during the replacement of the photodamaged PSII proteins. Pulse-labeling experiments of chloroplast-encoded proteins indicated that the synthesis of D1 was not affected in both wild-type and Δ*psbT* cells (data not shown). Thus we can conclude that a post-translational event(s) of the repair process is impaired in the absence of PsbT. However it remains unclear how PsbT facilitates the recovery of photodamaged PSII complex. In order to identify the recovery steps in which PsbT is specifically involved, further investigations on integration of newly synthesized proteins and cofactors into the photodamaged PSII complex would be necessary.

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