S22-030

Roles of the PsbL protein in electron transfer on the donor side of PSII

T Iwata[1], Y Tsuchida[1], T Shiina[2], and Y Toyoshima[1]

[1]*Graduate School of Human and Environmental Studies, Kyoto Univ., Yoshida-nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501 Japan*

[2]*Faculty of Human Environment, Kyoto Pref. Univ., Shimogamo-hangi-cho, Sakyo-ku, Kyoto 606-8522 Japan*

Keywords: Photosystem II (PSII), PsbL, donor side electron transfer, plastid transformation

Introduction

The photosystem II (PSII) complex utilizes light energy to oxidize water and reduce plastoquinone through a photosynthetic electron transport chain. When the light intensity exceeds the capacity of the chain, the absorption of excess energy results in the damage of the PSII complex. To minimize the damage, various aspects of light dependent regulation are suggested including the regulation of the electron transfer at the donor side and/or acceptor side of PSII. We reported that PsbL protein is involved in the donor side electron transfer of PSII through its carboxyl terminal region (Hoshida *et al*., 1997).

In this report, first, in order to examine the role of PsbL in the PSII function *in vivo*, we produced transplastomic tobacco plants lacking *psbL* gene by homologous recombination. In the resulting transformants, the PSII activity was not detected, suggesting PsbL to be essential for the functioning of PSII. Second, we examined the effect of pH on the electron transfer at the donor side of PSII under a speculation that it would be affected by the change of pH at lumen, which alters responding to light intensity. The donor side electron transfer was found to depend on pH. Two components (pK 4.5 and pK 6.5) are involved in the donor side electron transfer and PsbL supports the component with pK4.5.

Materials and methods

Transformation vectors

The transformation vectors pTY5 and pTY6 were designed to produce transgenic tobacco control (WT*) and *psbL* deleted (Δ*psbL*) plants. The DNA fragment containing the *psbEFLJ* operon was isolated as a fragment of *Sal*I-*Spe*I tobacco plastid DNA and inserted into a pBluescript II SK^+ plasmid (Stratagene) to produce the plasmid pTY1. Deletion mutation of the *psbL* gene was introduced into pTY1 by site-directed mutagenesis kit (TaKaRa) using a synthetic oligonucleotide 5'-TTAATC CGAATTATAGAGCTGAAAACGAAAGAGAATCAA-3' and the resulting plasmid was named pTY2. The *aadA* cassette was isolated from pCT08 (Shikanai *et al.,*1998) and cloned into the *Eco*RV site of pTY1 and pTY2 to give pTY5 and pTY6, respectively.

Plant, plastid transformation and plant regeneration

Tobacco (*Nicotiana tabacum* cv. Xanthi) plants were grown on a RM medium with or without sucrose (30 g/L) under 16h of light at 25˚C. Light was provided by fluorescent lumps at an intensity of 30 $\mu E \bullet m^{-2}s^{-1}$.
Transformation of tobacco plastids and selection of transgenic shoots were carried out as described by Shiina *et al*. (2000).

Disintegration and reconstitution of PSII reaction center complex

PSII core complex (original RC) and PsbL-depleted PSII complex (depleted RC) were isolated from spinach as described by Hoshida *et al.* (1997).
Reconstitution of depleted RC with PsbL was carried out as described by Ozawa *et al*. (1997) except for dialyzing at 8 ˚C.

Chlorophyll Fluorescence Measurement

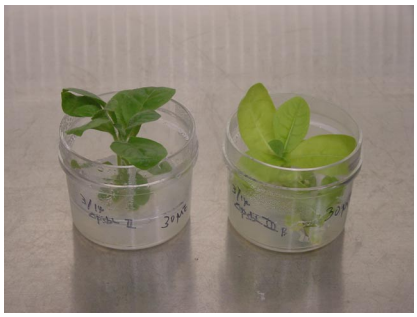
Chlorophyll fluorescence was measured using a pulse modulated amplitude fluorometer (PAM-2000, Walz). Modulation frequency of measuring beam was 600 Hz. A saturation pulse was given at 2000 $\mu E \bullet m^{-2}s^{-1}$ for 0.8sec. Actinic light of 30 $\mu E \bullet m^{-2}s^{-1}$ was provided by the LED lumps.

EPR Spectroscopy

X-band EPR measurements were carried out with a JES-FE2XG spectrometer (JEOL). Original RC, depleted RC and PsbL-reconstituted RC were suspended at concentration of 50μM Cytb-559 in a solution of 50mM glutamic acid (pH3.5-4.5), MES (pH4.5-7.0) TES (pH7.0-8.0) or CHES (pH8.0-9.0) containing 0.4M sucrose and 5mM ferricyanide.
EPR measurement conditions were as follows: microwave frequency, 9.43 GHz; microwave power, 1 mW; modulation frequency, 100 kHz; modulation amplitude, 3.2 G.

Results

To examine the role of PsbL in the PSII function *in vivo*, we introduced the deletion mutation of *psbL* into tobacco plants and obtained homoplasmic-transformed plants lacking PsbL (Δ*psbL*). For a control, we produced tobacco plants which contains *aadA* cassette into the spacer region downstream of the *psbEFLJ* operon (WT*). The Δ*psbL* tobacco did not grow photoautotrophically, while on sucrose-containing medium, they grew, but significantly slower than WT* and their leaves were pale green (Fig. 1).



Expanded leaves of WT* contain 1.0-1.5 μg of chlorophyll per gram wet weight, Δ*psbL* leaves contain ~0.3 μg chl/g leaves. The ratio of chlorophyll a to b in Δ*psbL* (2.0) was significantly lower than WT*(2.5-2.6).

Fig. 1. Deletion of *psbL* results in a pale green phenotype. WT* (left) and *ΔpsbL* (right) plants are shown.

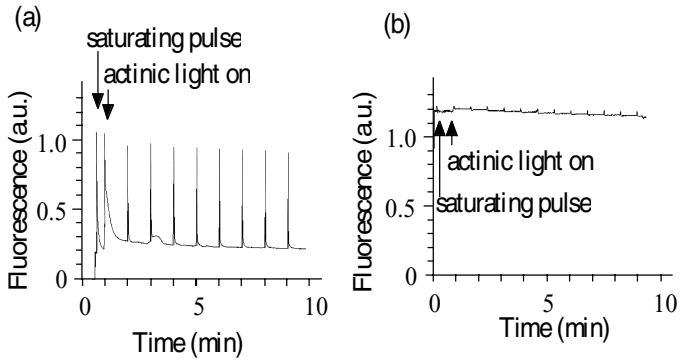


Fig. 2. Chlorophyll fluorescent yield measurements. (a) Chlorophyll fluorescent induction kinetics of dark-adapted WT* leaf. (b) Chlorophyll fluorescent induction kinetics of dark-adapted *ΔpsbL* leaf.

PSII activities were measured with tobacco leaves of WT* and Δ*psbL* by chlorophyll fluorescence. Δ*psbL* had high Fo fluorescence level as to be comparable to Fm of WT*, and maximal efficiency of PSII (Fv/Fm) was less than 0.1 while Fv/Fm of WT* was ~0.8 (Fig. 2). These results indicate that PSII does not function or PSII particle does not exist in Δ*psbL*.

We have shown that PsbL is involved in the donor side electron transfer in PSII by *in vitro* reconstitution experiments. As PsbL contains no redox component, we suggested that PsbL plays a role of regulation of electron transfer at the donor side of PSII. Since the donor side of PSII is located at the lumen, we speculate that the donor side of PSII would be affected by the change of pH in the lumen. To examine the effect of pH on the PsbL supported electron transfer at the donor side of PSII, the amounts of photoinduced tyrosyl radical were estimated by EPR measurements in spinach PSII core complex (original RC), PsbL-depleted PSII complex (depleted RC) and PsbL-reconstituted PSII complex (reconstituted RC).

Figure 3 summarizes the effect of pH on the formation of signal $II_{f/s}$. The amplitude of the signals increased in each RC as pH increased. The signal $II_{f/s}$ in the original RC showed two-step pH dependence with the pK values of 4.5 and 6.5, respectively. In depleted RC, however, the component with pK4.5 was completely disappeared. In reconstituted RC, the component with pK4.5 recovered.

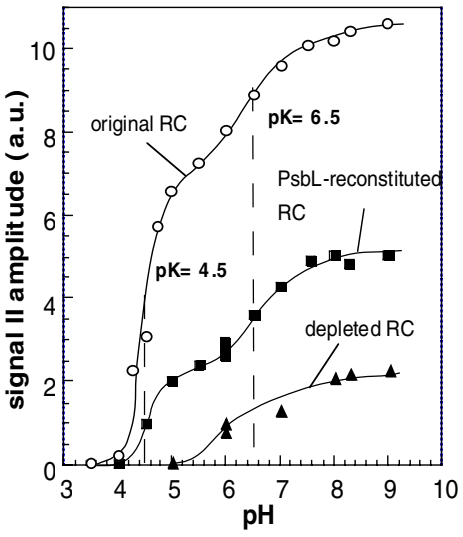


Fig. 3. pH dependence of the amplitudes of total signal II in RC (open circles), depleted RC (closed triangles) and reconstituted RC (closed squares) with PsbL. The formation of signal II was measured by EPR during continuous illumination at various pHs. The amplitudes were determined from the values at the low-field peak (g = 2.02).

These results show that the donor side electron transfer is affected by pH, and that two independent components are involved in the donor side electron transfer; one is the component pK4.5 which is supported by PsbL, another is the component pK6.5 which functions without PsbL.

Discussion

The deletion mutation of *psbL* gene causes the fatal damage to the PSII function in tobacco plants. There are two possibilities: Δ*psbL* might have no PSII particle. If it is, PsbL is essential for the formation of PSII complex; PSII complexes might exist in *ΔpsbL* but not function. If it is, PsbL plays a role of the regulation of electron transfer in PSII.
in vitro reconstitution experiments reveal that PsbL regulates the donor side electron transfer by sensing pH. This result supports the second possibility. PsbL may play an important role of the regulation of the electron transfer at the donor side of PSII by sensing the pH, which changes in a range from 4 to 6 by light intensity.
It is probable that PsbL functions as an adjustor of the electron transfer in PSII.

Acknowledgements

We thank Dr. T. Shikanai (Nara Institute of Science and Technology, Japan) for providing us with plasmid pCT08, Dr. T. Endo (Kyoto Univ., Japan) for helpful discussion and Prof. G. Takeba (Kyoto Prefectural Univ., Japan) for access to a PAM-2000 fluorometer.

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