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Dark-chilling Pretreatment Protects PSI from Light-chilling Damage

Hideki Kudoh and Kintake Sonoike*
Department of Integrated Biosciences, The University of Tokyo, Kashiwa 277-8562, Japan

In chilling-sensitive plants, the donor side of Photosystem II is inhibited by the chilling treatment in the dark, while the acceptor side of Photosystem I is inhibited by the chilling under the moderate light. Since the addition of inhibitors of electron transfer from Photosystem II protects Photosystem I from chilling induced photoinhibition of Photosystem I, inhibition or down-regulation of Photosystem II activity in vivo may also protect Photosystem I from photoinhibition. It was revealed that dark-chilling pretreatment actually protected Photosystem I from photoinhibition. The results imply that down-regulation of Photosystem II under stress conditions may have a role to protect Photosystem I from photoinhibition.

Key words : Photosystem I, Photosystem II, chilling, damage, temperature stress, photoinhibition

INTRODUCTION

Photosynthetic activities of chilling-sensitive plants such as cucumber, tomato or common bean are inhibited when they are exposed to low, but non-freezing temperatures [1]. In chilling-sensitive plants, oxygen-evolving activity is lost after long periods of chilling treatment in the dark [2, 3]. The site of inhibition by the chilling treatment of cucumber leaves in the dark is the water-splitting machinery of Photosystem (PS) II [4] due to the release Mn^{2+} from the oxidizing side of PSII [5]. Chilling under moderate light, however, causes photoinhibition of PSI in chilling-sensitive plants [6-9]. Although the damage to PSII by the chilling in the dark is reversible and the activity of PSII quickly recovered under illumination at room temperature [3], the inhibition of PSI due to chilling under light is irreversible [10]. Therefore, the damage to PSI must be more serious than that to PSII for the plant survival.

It must be noted that the inhibition of PSI is completely suppressed by the addition of DCMU or DBMIB, which blocks electron transfer from PSII to PSI [11, 12]. This observation implies that any kind of inhibition in PSII activity might protect PSI from photoinhibition. In this study, we investigated the suppressive effect of dark-chilling pretreatment on the photoinhibition of PSI.

MATERIALS AND METHODS

Plant materials. Cucumber (Cucumis sativus L. cv.

*To whom correspondence should be addressed.
E-mail: sonoike@k.u-tokyo.ac.jp

Nanshin) plants were grown hydroponically [13] at 30°C under conditions of 14 h of light (300 $\mu molm^{-2}s^{-1}$) and 10 h of darkness.

Dark-chilling and photoinhibitory treatment. Leaf discs were wrapped in aluminum foil and treated for 42 h at 0°C on ice. After the pretreatment, the leaf discs were chilled for 5 h by placing them on water at 4°C under the moderate light (250 $\mu mol\ m^{-2}s^{-1}$).

For the PSII photoinhibitory treatment, leaf discs were floated on 25°C water under strong illumination (7000 -14000 $\mu mol\ m^{-2}s^{-1}$) from a light source (Cold Spot PICL-NRX, NPI, Tokyo, Japan) for 10 min.

Determination of photooxidizable P-700. Thylakoid membranes were isolated from chilled and control leaves as described in Terashima et al. [13]. In order to determine the concentration of photooxidizable P-700, light-minus-dark difference absorption changes at 701 nm were measured using a spectrophotometer (model 356, Hitachi, Tokyo, Japan) [7, 8]. Chlorophyll concentration was determined after extraction with 80% acetone according to Porra et al. [14]. The absorption change around 830 nm due to P-700 oxidation was measured in vivo using a pulse-modulated system (PAM 101/102, Walz, Effeltrich, Germany) [15]. P-700 was oxidized by far-red light from a photodiode (FR-102, Walz Effeltrich, Germany). The intensity of the far-red light was 13.5 W m^{-2}.

Measurement of chlorophyll a fluorescence. Chlorophyll (Chl) fluorescence was measured using a PAM Chl fluorometer (PAM 101/102/103, Walz, Effeltrich, Germany). Leaf discs were dark-adapted for 5 min and then the measuring light (ML) was turned on to obtain the minimal fluorescence level (Fo). The maximum fluorescence level with fully reduced Q_A in dark-adapted leaf (Fm) was obtained by applying saturating pulse (5600 $\mu mol\ m^{-2}s^{-1}$) from a light source (KL 1500, Schott, Wiesbaden, Germany). Fv/Fm was calculated as (Fm-Fo)/Fm.

RESULTS AND DISCUSSION

When leaf discs of cucumber were treated at 4°C for 5 h under moderate light (250 $\mu molm^{-2}s^{-1}$), the amount of photooxidizable P-700 determined in isolated thylakoid membrane from the leaf discs was decreased to 20% of untreated level (Fig.1 untreated, filled bar). The decrease of photooxidizable P-700 was not observed in the sample treated at 30°C under light (Fig.1 untreated, open bar). This result supports the observations that PSI is photoinhibited at chilling temperature under moderate illumination [6-9].

Interestingly, however, the decrease of photooxidizable P-700 in chilled leaves under moderate light was suppressed

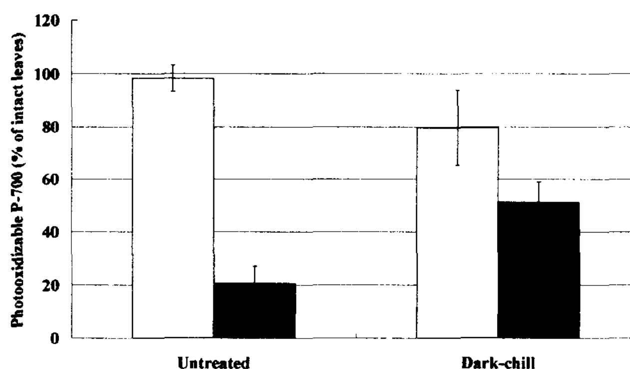

Fig.1 Effect of dark-chilling pretreatment on the inhibition of PSI. Photooxidizable P-700 content was measured *in vitro* using a spectrophotometer. Thylakoid membranes were isolated from the cucumber leaves treated at 4°C (filled) or at 30°C (open) for 5 h under light at 250 μmol photons $m^{-2}s^{-1}$ with (dark-chill) or without (untreated) pretreatment at 0°C in dark for 42 h. Error bar shows S.E. (n=3).

by the pretreatment of the leaf discs at 0°C for 42 h in the dark (Fig.1 Dark-chill, filled bar). The result clearly shows that photoinhibition of PSI induced by the chilling under moderate light is partially suppressed by the dark-chilling pretreatment of leaf discs. Thus, inactivation of PSII *in vivo* induced by the dark-chilling treatment actually protects PSI from photoinhibition. It is well known that D1 protein, one of the targets of PSII photoinhibition, is a rapid-turnover protein and that the photoinhibition of PSII can be rapidly recovered by the replacement of D1 [16]. On the other hand, the recovery of PSI from photoinhibition is very slow [10]. Thus the inhibition of PSI must be more harmful for plant survival than that of PSII. Inactivation of PSII under various stresses may be regarded as a protective mechanism of PSI from photoinhibition [17].

In order to check whether the photoinhibition of PSI is suppressed by the photoinhibition of PSII due to high light stress, we tried to induce selective photoinhibition of PSII by applying high light at 25°C. Maximum quantum yield of PSII gradually decreased with the increase of photon flux densities upon the treatment (Fig.2A, open bars). Unexpectedly, the amount of functional PSI also decreased by the strong light (Fig.2A, closed bars). Since the amount of photooxidizable P-700 determined *in vivo* was underestimated due to the enhancement of cyclic electron transfer around PSI just after stress [10], we also checked the P-700 content 24 h after the stress when the effect of cyclic electron transfer is supposed to be diminished. The considerable decrease of P-700 photooxidation is still observed after 24 h (Fig.2B, closed bars) so that PSI was apparently inhibited by high light stress. At least in cucumber, PSI seems to be as sensitive as PSII. Thus we could not induce selective photoinhibition of PSII and test if photoinhibition of PSII protects PSI from photoinhibition in

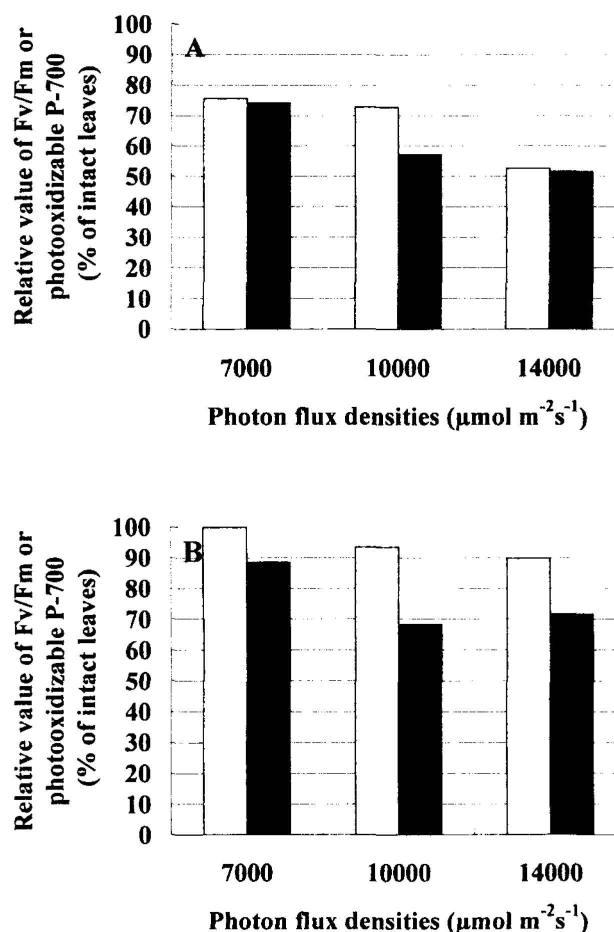

Fig.2 Effect of high photon flux density on Fv/Fm (open bars) and photooxidizable P-700 (closed bars), determined *in vivo* using a PAM fluorometer at 0 h (A) or at 24 h (B) after the illumination for 10 min at indicated photon flux densities.

this study. Using other species of chilling-sensitive plants as experimental material, it may be possible to prove the protecting effect of photoinhibition of PSII on the photoinhibition of PSI in near future.

REFERENCES

1. Lyons J.M. (1973) Chilling injury in plants. *Ann.Rev. Plant Physiol.* 24, 445-466.

2. Margulies M.M. (1972) Effect of cold-storage of bean leaves on photosynthetic reactions of isolated chloroplasts. Inability to donate electrons to Photosystem II and retention to manganese content. *Biochim. Biophys. Acta* 267, 96-103.

3. Kaniuga Z., B. Sochanowicz, J. Zabek, and K. Krzystyniak (1978) Photosynthetic apparatus in chilling-sensitive plants. I. Reactivation of Hill reaction activity on the cold and dark storage of detached leaves and intact plants. *Planta* 121, 121-128.

4. Terashima I., L-K. Huang, and C.B. Osmond (1989) Effects of leaf chilling on thylakoid functions, measured at room temperature, in *Cucumis sativus* L. and *Oryza sativa* L. *Plant Cell Physiol.* 30, 841-850.

5. Shen J-R, I. Terashima, and S. Katoh (1990) Cause for dark, chilling-induced inactivation of photosynthetic oxygen-evolving system in cucumber leaves. *Plant Physiol.* 93, 1354-1357.

6. Sonoike K. and I. Terashima (1994) Mechanism of Photosystem-I photoinhibition in leaves of *Cucumis sativus* L. *Planta* 194, 287–293.

7. Terashima I., S. Funayama, and K. Sonoike (1994) The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperature is Photosystem I, not Photosystem II. *Planta* 194, 287–293.

8. Sonoike K., I. Terashima, M. Iwaki, and S. Itoh (1995) Destruction of Photosystem-I iron–sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures. *FEBS Letters* 362, 235–238.

9. Tjus S.E., Møller B.L., and Scheller H.V. (1998) Photosystem I is an early target of photoinhibition in barley illuminated at chilling temperatures. *Plant Physiol.* 116, 755–764.

10. Kudoh H., and K. Sonoike (2002) Irreversible damage to Photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. *Planta* (in press).

11. Havaux, M., A. Davoud (1994) Photoinhibition of photosynthesis in chilled potato leaves is not correlated with a loss of Photosystem II activity. Preferential inactivation of Photosystem I. *Photosynth. Res* 40, 75–92.

12. Sonoike, K. (1995) Selective photoinhibition of Photosystem I in isolated thylakoid membranes from cucumber and spinach. *Plant Cell Physiol.* 36, 825–830.

13. Terashima I., Y. Kashino, and S. Katoh (1991) Exposure of leaves of *Cucumis sativus* L. to low temperatures in the light causes uncoupling of thylakoids I. Studies with isolated thylakoids. *Plant Cell Physiol.* 32, 1267–1274.

14. Porra R.J., W.A. Thompson, and P.E. Kriedemann (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* 975, 384–394.

15. Schreiber U., C. Klughammer, and C. Neubauer (1988) Measuring P-700 absorbance changes around 830 nm with a new type of pulse modulation system. *Z. Naturforsch.* 43c, 686-698.

16. 16.Aro E.M., I. Virgin, and B. Andersson (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochim. Biophys. Acta* 1143, 113-134.

17. Sonoike K. (1996) Photoinhibition of Photosystem I; its physiological significance in the chilling sensitivity of plants. *Plant Cell Physiol.* 37, 239-247.