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Inactivation of Cu/Zn-SOD precedes the degradation of photosystem I reaction center, PsaA/B, in cucumber leaves chilled in the light

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Introduction

Chilling (0 – 12 ºC) is common during the growing season in temperate regions and can substantially decrease plant productivity. Photosynthetic electron transport is viewed as a primary target site for chilling stress although the carbon reduction cycle and the control of stomatal conductance are principally impaired. In chilling-sensitive plants as in chilling-resistant plants, the combination of low temperature with high light enhances the induction of photodamage, resulting from the inhibition of photosynthetic capacity, repair mechanisms, xanthophyll-cycle-related energy dissipation, or antioxidant enzyme activity. While PSII is a photoinhibitory site for chilling resistant plants, PSI in chilling sensitive plants, at least in cucumber leaves, is regarded as the main site for photoinhibition at chilling temperatures (for a review, see Sonoike 1998). However, the evidence that PSI activity declines to a greater extent than PSII is not necessarily sufficient to identify PSI as a primary target of chilling. This is because it does not exclude the possibility that downstream chilling-susceptible processes (carbon metabolism and stomatal conductance) and/or the antioxidant systems are the primary targets. Consequently, there is insufficient evidence from intact leaves of chilling sensitive plants to classify PSI as a primary target of light-chilling stress. In the present study, we addressed the possibility that a particular component of the ROS-scavenging system that is primarily inactivated prior to PSI degradation. Experiments using chilling-sensitive cucumber leaves showed that the rapid increases in leaf H_2O_2 contents might play a role in the inactivation of Cu/Zn-SOD and hence the photoinactivation of PSI.

Materials and methods

Cucumber (*Cucumis sativus* L. cv Eunhwa) grown in growth chambers (14 h light/28°C; 10 h dark/25°C) under fluorescent light with light intensity of 100 μmol m^{-2} s^{-1} at the upper surface of leaves. Leaf disks (each 0.79 cm^2) from the youngest fully expanded pair of leaves of 20 - 22 days old plants were floated on distilled water and exposed to low temperatures (5 °C) for 6 h at 100 μmol m^{-2} s^{-1}.

The maximum efficiency of PSII was estimated from the chlorophyll fluorescence ratio, Fv/Fm, at room temperature using Hansatech Plant Efficiency Analyzer. Light-response curves of photosynthetic O_2 evolution during illumination were determined with a leaf-disc O_2 electrode (Oxygraph system, Hansatech) in air with 5% CO_2 at 25°C. The redox state of P700 leaf disk was determined *in vivo* using a PAM-101 modulated fluorometer (Heinz Walz GmbH, Effeltrich, Germany) equipped with ED P700DW emitter-detector and PAM Data Acquisition System (PDA-100).

PSI reaction center PsaA/B were measured immunochemically after isolation of thylakoid membrane. Isozymes of superoxide dismutase and ascorbate peroxidase and activity staining were carried out according to Baum and Scandalios (1979) and Mittler and Zilinskas (1993), respectively. H_2O_2 content was measured according to the modified method of Bernt and Bergmeyer (1974) using peroxidase enzyme.

Results

Photosynthetic performance of cucumber leaves exposed to light chilling stress was severely retarded. Maximal photosynthetic O_2 evolution at saturating light intensities (Pmax) in the leaves maintained at 5°C decreased to about 35% of control leaves at 20 °C, resulting in the typical photoinhibition phenomena of photosynthesis (Table 1). In order to see that this light-chilling-induced photoinhibition of photosynthesis in cucumber is closely related to photoinhibition of PSI rather than PSII, we determined the quantum yield of PSII and photooxidizabe P-700 in vivo. As shown in Table 1, photoinhibition of PSII in cucumber leaves decreased by less than 7%, but that of PSI decreased by 82 %. Thylakoid membranes isolated from the light-chilled leaves and PSI reaction center protein PsaA/B polypeptides were quantified by immunoblot analysis. As shown in Fig 1, no pronounced changes in the reaction center protein were noticeable in leaves exposed to low light illumination at various temperatures.

Table 1. Light chilling-induced changes in maximal photosynthesis (Pmax), photochemical yield of PSII (Fv/FM), the photoxidizable $P700^+$ (1×10^{-3}) and H_2O_2 content in cucumber leaves

Tem. (°C)	Pmax (μmol O_2 mg chl h^{-1})	Fv/Fm	$\Delta A/A_{820}$ (1×10^{-3})	H_2O_2 (μmol m^{-2})
20	14.93 ± 0.66	0.83 ± 0.03	3.30 ± 0.41	1.62 ± 0.24
5	9.63 ± 0.59	0.77 ± 0.01	0.57 ± 0.05	5.32 ± 0.14

Scavenger enzymes Cu/Zn-SOD and APX effectively scavenge active oxygen species generated in chloroplasts and hence their activities were investigated using gel assay systems. As shown in Fig. 2A, only chloroplastic Cu/Zn-SOD activity was decreased by approximately 80% compared to the control leaves at 20°C. However, low temperature by itself (dark-chilling) hardly induced any changes in any SOD activities investigated (Fig. 2B). Interestingly, light chilling hardly affected active-oxygen scavenging enzymes of ascorbate peroxidase (Fig. 2C) despite its protective role in the scavenging process. This inactivation of Cu/Zn-SOD activity is likely attributable to the increase in leaf H_2O_2 contents (Table 1).

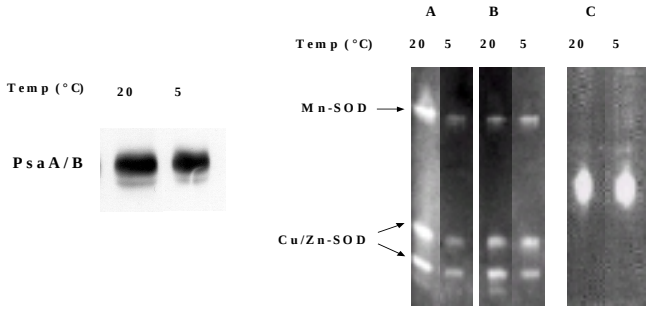

Fig. 1. *Left*, Representative immunoblots of PSI reaction center PsaA/B heterodimer from the cucumber leaves exposed for 6 hrs at 5 and 20°C.

Fig. 2. *Right*, Zymograms for superoxide dismutases (A and B) and ascorbate peroxidase (C) from the cucumber leaves exposed for 6 hrs at 5 and 20°C with (A and C) or without (B) low light illumination.

Discussion

Chilling stress in cucumber leaves induced the photoinhibition of photosynthesis, resulting in a decline in photosynthetic oxygen evolution, which is mainly mediated by inactivation of PSI, confirming PSI as a primary target site of light chilling stress in the chilling-sensitive plants. However, in the present study, the PsaA/B reaction center protein of PSI was hardly fragmented (Fig. 1) despite dramatic decreases in photooxidisable P700 (Table 1), suggesting that dysfunction in PSI photochemistry occurs well before the degradation of reaction center proteins.

The inhibition of PSI in cucumber leaves appears to be due to the inactivation of PSI protective mechanisms. In order to look for the most probable candidate for chilling–sensitive step, SOD and APX activities were investigated. APX activity was not affected by light-chilling stress, but Cu/Zn-SOD was. However, no detectable SOD decay was observed in leaves kept in the dark for 6 hr. In chloroplasts, subcellular components including SOD, APX and PSI reaction centers are inactivated by various active oxygen species (Asada 1999) and hence, it is expected that leaf hydrogen peroxide contents would be increased by light chilling. As expected, about 3 fold increase in H_2O_2 content was indeed observed, strongly suggesting the involvement of H_2O_2 in the inactivation processes of PSI and Cu/Zn-SOD.

In conclusion, we propose that when cucumber leaves are exposed to light chilling stress, the increased concentration of H_2O_2, which is not catalyzed by Cu/Zn-SOD, inactivates plastidic Cu/Zn-SOD. With the loss of ability to dismutate O_2^-, highly reactive $^{\bullet}OH$ could be formed from O_2^- and H_2O_2. Under such conditions photoprotection of PSI by Cu/Zn-SOD would be dysfunctional, resulting in the preferential inactivation of PSI relative to PSII. Hence, the most chilling-sensitive site in cumber leaves will be most likely to be the chloroplastic Cu/Zn-SOD.

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