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Reduction of the Mn cluster in the oxygen evolving complex of cucumber leaves under dark-chilling condition

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Introduction

Photosynthetic oxygen evolution takes place at a tetranuclear Mn-cluster located in photosystem II (PSII). The functional Mn-cluster is known to include high oxidation states (III, IV) of Mn ions. Disintegration and photoassembly of the Mn-cluster have been extensively studied *in vitro*. For instance, isolated PSII membranes were treated with hydroxylamine to inhibit oxygen evolution, and the activity was restored by illumination in the presence of Mn ions. However, such inhibition and photoactivation processes have not been studied well *in vivo*. In particular, changes in the redox state of the Mn-cluster during those processes *in vivo* have not been characterized at all.

Oxygen evolution of cucumber leaves is known to be inhibited by chilling stress. The inhibition is reversible and the oxygen-evolving activity of dark-chilled leaves is fully restored after illumination at room temperature (Kaniuga *et al*., 1978). Thus, cucumber leaves with reversible inhibition by dark-chilling treatment will be a good model system to study the processes of destruction and photoassembly of the Mn-cluster *in vivo*. In this study, we have investigated the state of the Mn-cluster in the process of dark-chilling inhibition of cucumber leaves by means of thermoluminescence (TL) measurements.

Materials and Methods

Cucumber plants (*Cucumis sativus* L. cv Nanshin) were grown hydroponically in a controlled environment [14 h light (300 μmol m^{-2} s^{-1}) and 10 h dark at 30°C]. Fully expanded leaves were used in experiments. For dark-chilling treatment, detached leaves were placed on ice (~0°C) in the dark. Thylakoid membranes were prepared as described by Shen *et al*. (1990). For TL measurements, cucumber leaves were excited by saturating xenon flashes with an interval of 1 s at 10°C, and then rapidly frozen using cold N_2 gas. TL glow curves were measured with a heating rate of 40°C/min. The rate of oxygen evolution was determined at 25°C with a Clark-type oxygen electrode in the presence of 0.2 mM 2,6-dichloro-p-benzoquinone as an electron acceptor. The assay mixture contained 50 mM Mes/NaOH (pH 6.5), 0.4 M sucrose, 10 mM NaCl and 5 mM $MgCl_2$.

Results and Discussion

Figure 1 shows the oxygen evolving activities of cucumber leaves that were treated at 0°C in the dark for 0 to 48 h. The oxygen evolving activity decreased with longer dark-chilling treatment (Figure 1; circle) as described previously (Shen *et al.*, 1990). The activity of the leaves dark-chilled for 24 h was mostly restored when the leaves were illuminated (80 μmol m^{-2} s^{-1}) at room temperature for 30 min. However, much less reactivation was observed in the leaves treated for 48 h (Figure 1; triangle). Thus, there seems to be two types of inhitibion: reversible and irreversible.

To further characterize the state of the Mn-cluster during the process of dark-chilling inhibition, TL measurements were performed. Figures 2A and 2B show TL glow curves of control (untreated) and 24 h-treated leaves, respectively. In the control leaves, TL bands were observed at about 30°C upon a series of flash illumination. These are so-called B-bands, which arise from charge recombination between either the S_2 or the S_3 state of the Mn-cluster and Q_B^-. In contrast to the control leaves, TL bands were hardly detected upon one to three flashes in the 24 h-treated leaves. However, upon four-flash illumination, a B-band-like signal was observed at ~30°C.

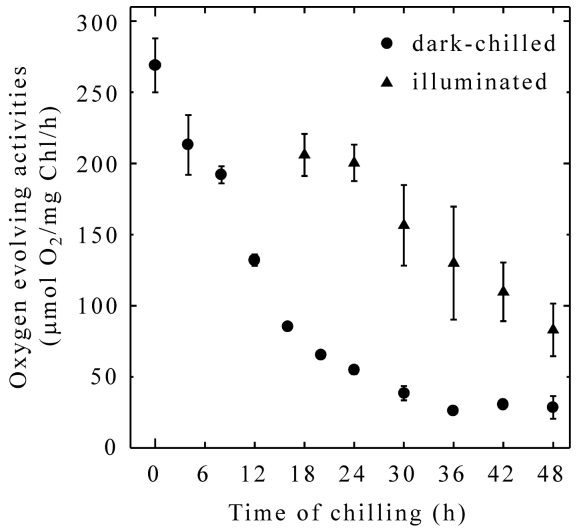

Figure 1. Effect of dark-chilling treatment on the oxygen evolving activity of cucumber leaves. Leaves were incubated at 0°C in the dark (circle) and then illuminated (80 μE/m^2 s) at room temperature for 30 min (triangle).

Figure 2C shows flash-number dependence of the TL intensity. The TL intensity of the B-band of untreated leaves showed maxima at the second and the sixth flashes, being typical of a period-four oscillation pattern of the S-state cycle. Dark-chilling treatment on cucumber leaves significantly altered this oscillation pattern. The prominent B-band intensity was observed after fourth and fifth flashes for the leaves dark-chilled for 24 and 36 h, respectively. This result suggests that the Mn-cluster was mostly in the S_{-2} and S_{-3} states after 24 and 36 h

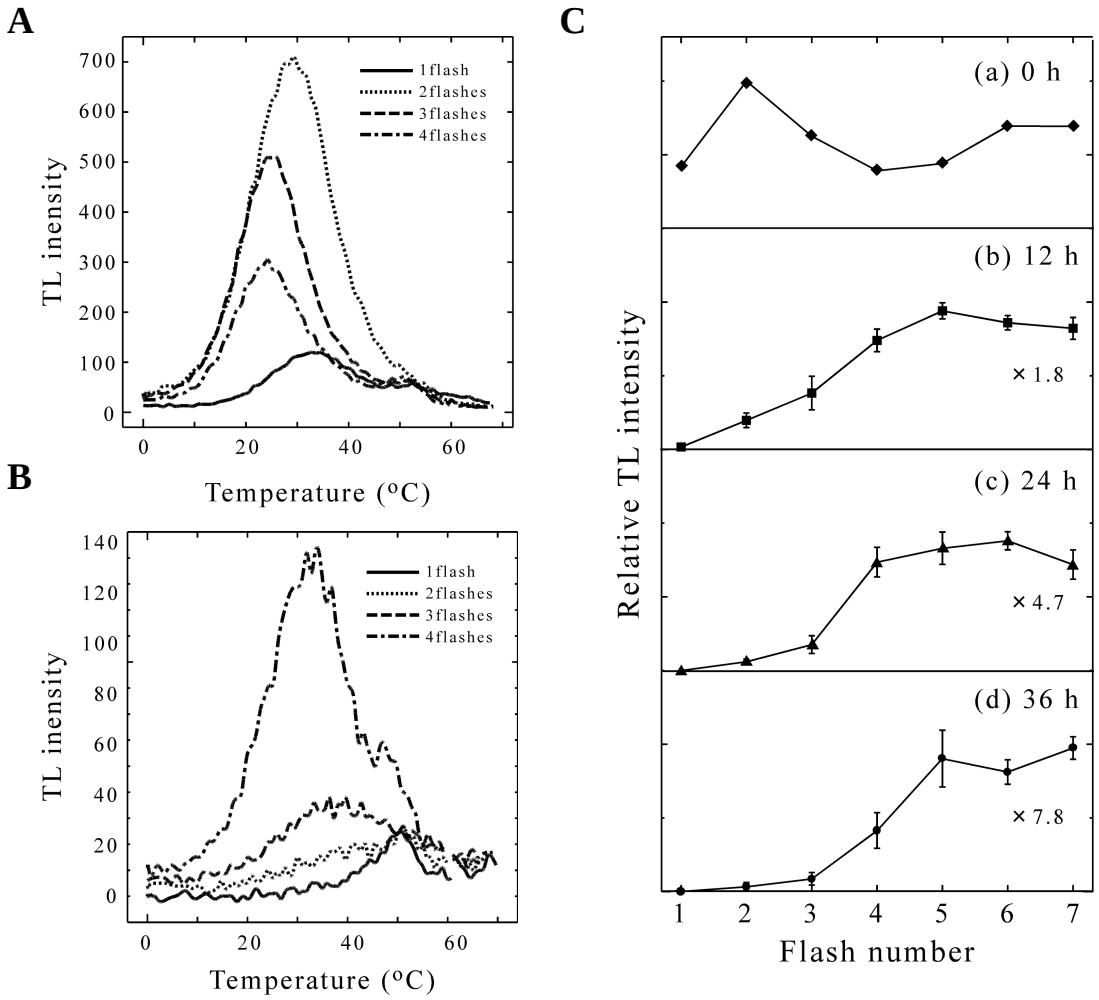

Figure 2. (A, B) TL glow curves of untreated cucumber leaves (A) and the leaves dark-chilled for 24 h (B) (C) Flash-number dependence of TL intensity for the leaves dark-chilled for 0, 12, 24, and 36 h. In C, data of at least four independent experiments were averaged.

of dark-chilling treatment, respectively. Low concentration of hydroxylamine has been reported to reduce the Mn-cluster *in vitro*, leading to formation of the S_{-1}, S_{-2} and S_{-3} states (Bouges, 1971; Messinger et al., 1997; Riggs et al., 1996). Similar over-reduction of the Mn-cluster must be induced by the dark-chilling treatment of cucumber leaves *in vivo*. Since the leaves dark-chilled for >12 h did not show an obvious oscillation pattern, it is considered that the Mn-cluster in the treated leaves may not exist in a single redox state but in a mixture of different redox states.

As shown in Figure 2C, the peak height of the B-band decreased to 56, 22 and 13% of that of the untreated leaves after dark-chilling treatment for 12, 24 and 36 h, respectively. The result suggests that the Mn-cluster is disintegrated as a consequence of over-reduction. The oxygen evolving activities decreased to 50, 20 and 10% after dark-chilling treatment for 12,

24 and 36 h, respectively (Figure 1), well corresponding to decrease in the B-band intensity. Thus, the cause of reversible inhibition of oxygen evolving activity is probably release of Mn ions from PSII.

In this study, we have observed the over-reduced states of the Mn-cluster, the S_{-2} and S_{-3} states, along with release of Mn ions, in cucumber leaves during dark-chilling inhibition *in vivo*. Such over-reduced forms might also be involved in the photoactivation process as precursors of the functional Mn-cluster.

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