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Destruction of photosystem I iron-sulfur centers in leaves of *Cucumis sativus* L. by weak illumination at chilling temperatures

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Abstract **The activity of photosystem (PS) I in cucumber leaves was selectively inhibited by weak illumination at chilling temperatures with almost no loss of P-700 content and PSII activity. The sites of inactivation in the reducing side of PSI were determined by EPR and flash photolysis. Measurement by EPR showed the destruction of iron-sulfur centers, F_X, F_A and F_B, in parallel with the loss of quantum yield of electron transfer from diaminodurene to $NADP^+$. Flash photolysis showed the increases in the triplet states of P-700 and antenna pigments, along with the decrease in the electron transfer from P-700 to F_A/F_B. This indicates the increase in the charge recombination between $P\text{-}700^+$ and A_0^-. It is concluded that weak-light treatment of cucumber leaves at chilling temperature destroys F_X, F_A and F_B and possibly A_1. This gives the molecular basis for the mechanism of selective PSI photodamage that was recently reported [Sonoike and Terashima (1994) Planta 194, 287–293].**

Key words: Photoinhibition; Chilling stress; Photosynthesis; Photosystem I; Reaction center; Iron-sulfur center

1. Introduction

Exposure of plants to excess light induces the inactivation of photosynthesis, especially when stresses other than light (e.g. chilling, high temperature or water stress) are overlaid [1]. The major target site has been known to be photosystem (PS) II [1,2]. However, we recently showed a selective photoinhibition of PSI in vivo by illumination at chilling temperatures [3,4]. When cucumber leaves were illuminated by a light of moderate intensity (220 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 4°C, the maximum quantum yield of electron flow through PSI (diaminodurene to $NADP^+$) decreased to 20–30% of the control value, with only a small inhibition of PSII activity [3]. The sequence of electron flow in PSI is considered to be as follows: $P\text{-}700 \rightarrow A_0 \rightarrow A_1 \rightarrow F_X \rightarrow F_A/F_B$, where A_0 represents the primary acceptor chlorophyll *a*, A_1 the secondary acceptor phylloquinone, and F_X, F_A and F_B the iron-sulfur centers (for a review, see [5]). The component(s) in the PSI acceptor-side between A_0 and F_A/F_B seems to be inactivated by the weak illumination at first, and then the destruction of P-700 and the degradation of the chlorophyll-binding large subunits (*psaA/B* gene products) seem to follow [4].

PSI has been reported to be inactivated by strong light under in vitro conditions. Satoh showed the inactivation of PSI as well as PSII in isolated spinach chloroplasts [6,7]. Satoh and Fork showed PSI inactivation in intact chloroplasts isolated from *Bryopsis* [8]. The photoinactivation site has been assigned to P-700 [8] or to be in the vicinity of the PSI reaction center [9]. Inoue et al. revealed the photodestruction of the PSI iron-sulfur centers under aerobic conditions [10], and the inhibition of electron transfer between A_0 and F_X after anaerobic illumination in isolated spinach thylakoid membranes [11]. In these in vitro experiments, PSI photoinhibition was usually induced by illumination at very high photon flux densities of more than 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The relationship between these high-light photoinhibitions in vitro and our weak-light photoinhibition in vivo is not clear yet.

In this study, the site of the inactivation in PSI in vivo in leaves of *Cucumis sativus* L. was determined to be the iron-sulfur centers and phylloquinone by flash photolysis and cryogenic EPR measurements.

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSI, photosystem I; PMS, phenazine methosulfate; P-700 and A_0, primary electron donor and acceptor chlorophylls; A_1, the secondary electron acceptor phylloquinone; F_X, F_A, F_B, iron-sulfur centers.

2. Materials and methods

Leaves of cucumber plants (*Cucumis sativus* L. cv. Nanshin) were prepared and treated at 4.3°C at various irradiance levels, and then thylakoid membranes were isolated from the treated leaves as described previously [3,12].

EPR signals were measured with a Bruker EPR-200 X-band spectrometer using an Oxford Instruments ESR-900 continuous liquid helium flow cryostat at the Analytical Center of National Institute for Basic Biology as described previously [13]. Samples (750 μM Chl) were suspended in 50 mM CHES-NaOH (pH 10), illuminated in the presence of dithionite and methyl viologen with a white light from a 150 W iodine-tungsten lamp of a slide projector for about 2 min at room temperature, and frozen in liquid nitrogen under illumination.

Flash induced absorption changes were measured by a split beam spectrophotometer as described previously [13]. Samples in a 1 × 1 × 4 cm plastic cuvette were illuminated by a 532 nm, 10 ns flash light from a frequency doubled Nd-YAG laser at a repetition rate of 0.5 or 1 Hz. 16–124 signals were averaged for each case as required. The basic reaction mixture contained 15 μM Chl, 50 mM Tris-HCl (pH 7.5), 10 mM sodium ascorbate, 40 μM PMS, 10 μM DCMU and 0.02% dodecyl maltoside. The concentration of PMS was increased to 80 μM for measurements in the μs time range. Before the measurements of absorption changes, reaction mixtures were centrifuged for 1 min at 15,900 × *g* to remove starch. Concentrations of chlorophyll were determined according to [14].

3. Results and discussion

3.1. Degradation of FeS centers determined by EPR

It has been suggested that some components on the acceptor side of PSI were inactivated by the light-chilling treatment of

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cucumber leaves, since the treatment suppressed PSI electron flow with small changes of P-700 content and PSII activity [4]. We determined the amount of iron-sulfur centers by EPR in thylakoid membranes isolated from leaves treated at 4.3°C. The membranes were frozen in the light in the presence of dithionite at pH 10 to fully reduce F_A/F_B and F_X. In the untreated sample, g = 1.94 (F_A), g = 1.92 (F_B), g = 1.89 (F_A/F_B mixture) and g = 1.78 (F_X) signals are clearly observed (Fig. 1, trace a). In the sample from dark-chilled leaves, similar amplitudes of the signals were observed (Fig. 1, trace b). A small difference might be due to the slight differences in the reduction level of F_A/F_B or F_X. In the sample from leaves treated at a photon flux density of 71 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 5 h, the magnitude of the F_A, F_B and F_X signals decreased to 53%, 66% and 52% (average of two measurements), respectively, of the control value (Fig. 1, trace c). When photon flux density was raised to 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the signal intensities of F_A, F_B and F_X decreased to 42%, 49% and 21%, respectively, of the control values (Fig. 1, trace d). It seems that the signal intensity of F_A/F_B and F_X decreased roughly in parallel with each other during the light-chilling treatments, although it is difficult to determine the contents of each iron-sulfur center accurately because of the presence of interacting forms of F_A/F_B.

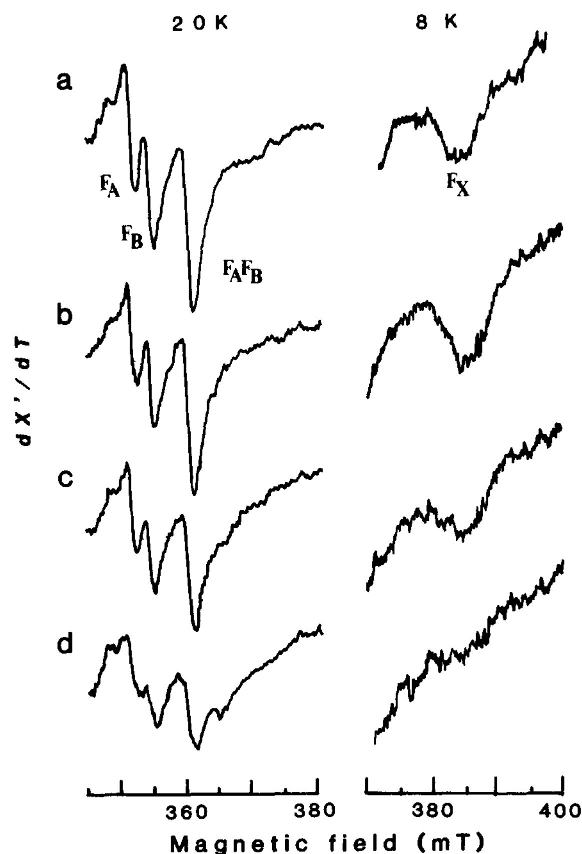

Fig. 1. EPR signals of photochemically reduced iron-sulfur centers in thylakoid membranes from untreated leaves (trace a), or from leaves treated at 4.3°C in the darkness (trace b), in the light at 71 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (trace c), or at 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (trace d). Microwave frequency, 9.63 GHz; scan time, 50 s. Microwave power, modulation amplitude and receiver gain were 20 mW, 10 G and 5×10^5 at 20 K; and 100 mW, 40 G, and 5×10^4 at 8K, respectively.

Fig. 2. Flash-induced absorption change at 701 nm (traces a, b) or at 432 nm (traces c, d) of thylakoid membranes from control leaf (traces a, c) or from leaves treated at 4.3°C in the light at 71 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 5 h (traces b, d). Flashes were fired at 1 Hz, and 128 signals measured with a resolution of 80 μs (traces a, b) or 1024 signals with a resolution of 3 μs (traces c, d) were averaged. Chlorophyll concentrations were 18 μM (traces a, b) or 9 μM (traces c, d).

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3.2. Kinetics of optical absorption change

When the thylakoid membranes isolated from the untreated cucumber leaves were excited by the laser flash, the absorption decrease due to photooxidation of P-700 was observed at 701 and 430 nm (Fig 2, traces a,c). The electron is known to go from P-700 to F_A/F_B through A_0, A_1 and F_X. (F_A/F_B^- then re-reduces P-700$^+$ with a half time of about 40 ms [15]. We added PMS, an artificial electron donor, to get full recovery of P-700 between repetitive flash excitations so that P-700$^+$ was mainly reduced by PMS with a half time of about 10 ms. The spectrum of the 10 ms phase showed a negative peak at 432 nm and a small positive peak at 450 nm, suggesting P-700$^+$ to be its major origin and $(F_A/F_B)^-$ to be a small contribution (data not shown, but see below).

If F_A/F_B is destroyed, a back electron transfer from F_X^- to P-700$^+$ with a half time of about 1 ms [5] can be detected. In the thylakoid membranes isolated from leaves that were illuminated at 4.3°C for 5 h at 71 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the magnitude of the slow phase decreased to about a half (Fig. 2, trace b) and the decay with a half decay time of 10–20 μs increased (Fig. 2, trace d), which could not be detected in the ms time range (Fig. 2, trace b). No decay component corresponding to the back electron transfer from F_X^- was detected. The slow phase gave a similar spectrum to that observed in thylakoids from untreated leaves, indicating the turnover of F_A/F_B in half of the PSI reaction centers.

The fast component (10–20 μs) showed a difference spectrum that indicates an absorption change of chlorophyll (decrease in the 430 nm region) and carotenoids (shift type change in the 460–480 nm region) (Fig. 3). A part of the fast phase may be attributed to the decay of the triplet state of antenna pigments, but not all, because A_0 was not destroyed by the photoinhibitory treatment, judging from the enhancing effect of methyl viologen on the photochemically determined P-700 [4]. So, the P-700 triplet produced by the charge recombination between P-700$^+$ and A_0^- may be attributed as well. The yield of the triplet by the charge recombination between P-700$^+$ and A_0^- is suggested to be about 30% at room temperature [16]. The extent of the fast decay component is almost the same as that of the slow component in the photoinhibited preparation (Fig. 2, trace d). Therefore, two-thirds of the fast absorption change seems to be ascribed to the triplet states of antenna pigments and one-third to the triplet state produced by the charge recombination. This suggests that the energy transfer between antenna pigments was also damaged by the photoinhibitory treatment. Another explanation for the fast decay components is the P-700$^+$ triplet state that was produced in the charge recombination reaction between P-700$^+$ and A_1^-. This reaction was also supposed to give the P-700$^+$ triplet state with an efficiency larger than 90% when the iron-sulfur centers were reduced chemically [17]. One more possibility is that the charge recombination between P-700$^+$ and A_1^- occurs directly to the ground

state of P-700$^+$ as in the photosystem I reaction center complex devoid of F_X and F_A/F_B [18]. At present we favor the first explanation since the contribution of the antenna pigments other than P-700$^+$ is obvious in the difference spectrum of the fast decay phase as shown in Fig. 3.

3.3. Parallel decrease in the FeS contents and the electron transfer rate

When the extents of the 10 ms decay phase of the flash induced absorption change in Fig. 2 (traces a, b) were plotted against the photon flux densities during the light-chilling treatment, they decreased almost in parallel with the amounts of iron-sulfur centers, measured by EPR, and with the relative quantum yields of PSI, measured by the activity of electron flow from the ascorbate-diaminodurene couple to NADP$^+$ reported earlier [4] (Fig. 4). The content of P-700$^+$ that can be determined by the chemically reduced-minus-oxidized difference spectra, on the other hand, did not decrease by these treatments [4]. We, thus, concluded that the light-chilling treat-

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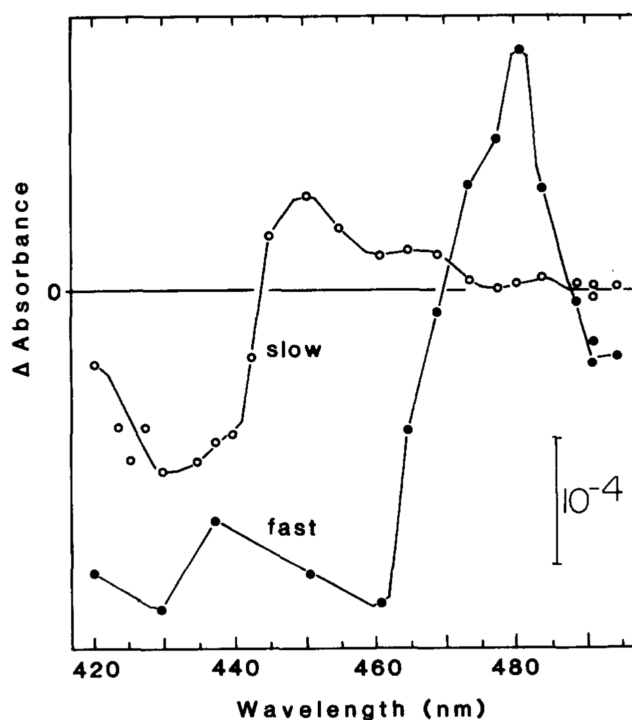

Fig. 3. The spectrum of flash induced absorption change of the thylakoid membranes isolated from photoinhibited leaves. Difference spectrum at 150 μs (slow) or between those at 7.3 μs and 50 μs after the flash excitation (fast) were plotted against wavelength. Experimental conditions are similar to those in Fig. 2.

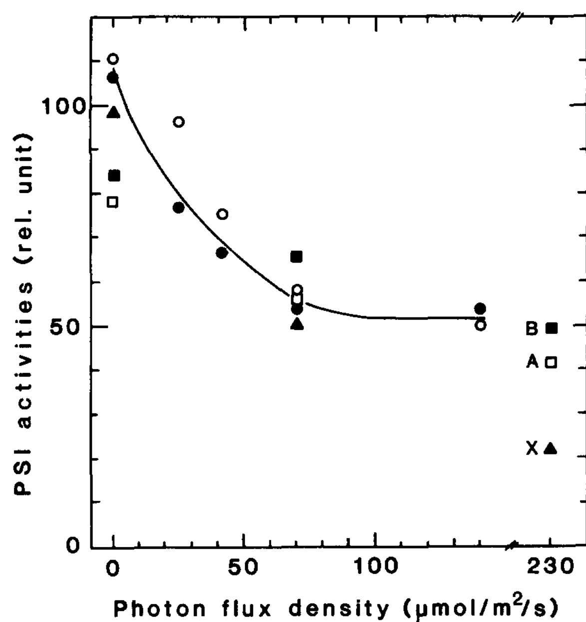

Fig. 4. Effects of photon flux density during the photoinhibition on the extent of the 10 ms-decay phase of the flash induced absorption change (filled circle), on the relative quantum yield of the $NADP^+$ reduction by reduced diaminodurene (open circle) and on the extent of F_A^- (open square), F_B^- (filled square) and F_X^- (filled triangle) signals determined by EPR. For the conditions of the measurements, see Figs. 1 and 2. The data of relative quantum yield are taken from [3].

ment induces parallel damage of F_X, F_A/F_B and A_1 without inactivation of P-700^+ and A_0.

The feature of the in vivo photodamage presented here somewhat differs from the photodamage produced by the strong illumination of PSI particles in vivo by Inoue et al. [10]. They observed that the extent of destruction of each iron-sulfur center was smaller than the decrease in $NADP^+$ photoreduction activity, and explained the latter activity to be the product of the remaining fractions of three iron-sulfur centers [10]. The in vivo photodamage observed in this study suggests the parallel damage of iron-sulfur centers, A_1 and $NADP^+$ reduction activity as shown in Fig. 4. Our working hypothesis at present, therefore, is that the PSI photodamage occurs in either F_A/F_B, F_X or A_1 (phylloquinone) at first, and then results in the inactivation of the other electron acceptors. It is not clear whether the partial damage of antenna pigments suggested in the present work are related to this inactivation process. The mechanism for the destruction of P-700^+, A_0 and the whole PSI reaction center complex, that occurs after stronger illumination, remains to be studied.

Sonoike and Terashima [4] showed that in vivo PSI photoinhibition requires the presence of oxygen. The oxygen radicals produced by the reducing power accumulated in the PSI electron acceptors may trigger the inhibition. Sonoike recently indicated that the PSI photoinhibition can be induced in vitro in isolated thylakoid membranes of spinach, as well as cucumber, if the reducing power is accumulated in the PSI acceptor side aerobically (paper submitted). Interestingly, in vitro photoinhibition of PSI occurred irrespective of the temperature during the treatment. The precise molecular mechanism of the in vivo PSI inhibition and its relationship to the well-known PSII photoinactivation will also be tested in the newly developed in vitro experimental system.

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