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Photoinhibition of photosystem I

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Introduction

P-700, the reaction center chlorophyll of PSI, was discovered by Kok (1956b). In the same year, Kok observed the decrease of photosynthesis by excess light, and named the phenomenon as "photoinhibition" (Kok 1956a). In early days, both PSI and PSII were reported as the site of inhibition (e.g. Satoh, 1970a, b, c). However, the subsequent studies revealed that the inhibition of PSII was usually greater than that of PSI at least *in vivo*. In the 1980s, the term photoinhibition became gradually a synonym for "photoinhibition of PSII". In a review article in 1984, Powles stated that the main site of photoinhibition is PSII (Powles 1984) and the statement was widely accepted among photosynthesis researchers. A few cases that reported the photoinhibition of PSI *in vitro* (Satoh and Fork 1982, Inoue et al. 1986, 1989) tended to be considered as an artificial phenomenon. In 1994, however, the first evidence for a selective photoinhibition of PSI *in vivo* was reported (Terashima et al. 1994, Havaux and Davaud 1994, Sonoike and Terashima 1994). Since then, a number of papers reporting the photoinhibition of PSI were published. Now, it is established that PSI could be a target of photoinhibition as well as PSII. In this article, the recent advances in the study of PSI photoinhibition including some new results from our laboratory are overviewed.

Materials and Methods

Cucumber (*Cucumis sativus* L. cv. Nanshin) plants were grown hydroponically (Terashima et al. 1991) at 30˚C under conditions of 14 h of light (190 μmol $m^{-2}s^{-1}$) and 10 h of darkness. Attached leaves were chilled for 5 h by placing them on water at 4˚C under the light at 190-200 μmol $m^{-2}s^{-1}$. For 1,3-dicyclohexyl carbodiimide (DCCD) treatment, the leaves were infiltrated with aqueous solution of 200 μM DCCD. The absorption change around 830 nm due to P-700 oxidation was determined *in vivo* using a pulse-modulated system (PAM 101/102, Walz, Effeltrich, Germany). Thylakoid membranes were isolated from photoinhibited and untreated leaves as described in Terashima et al. (1991). To determine photo-oxidizable P-700 *in vitro*, light minus dark difference absorption changes at 701 nm were measured using a spectrophotometer (model 356, Hitachi, Tokyo, Japan) (Terashima et al. 1994, Sonoike 1995). Chlorophyll concentration was determined after extraction with 80% acetone according to Porra et al. (1989). The proteins of the thylakoid membranes were resolved by SDS-PAGE using 16-22% acrylamide gel containing 7.5 M urea (Ikeuchi and Inoue 1988). Western blotting was performed by electrophoretic transfer of proteins to polyvinylidene difluoride (PVDF) membrane (Millipore, Milwaukee, WI, USA). The reaction with antibodies was carried out as described in Kashino et al.

(1990). The antiserum against the PsaA/B polypeptides from *Thermosynechococcus elongatus* strain BP-1 (formerly *Synechococcus elongatus* strain BP-1) was kindly provided by Dr. I. Enami. It was demonstrated that this antiserum against the proteins from *Thermosynechococcus elongatus* has a high cross-reactivity to the proteins from higher plants (Kashino et al. 1990). The band images were scanned with an image scanner (GT-7000U, EPSON, Tokyo, Japan). The relative intensity of each band was estimated by densitometry using NIH image software.

Results and Discussion

1. Generality of the phenomenon

Initially, the photoinhibition of PSI was mainly reported in chilling sensitive plants such as cucumber (Terashima et al. 1994), common bean (Sonoike et al. 1995b) or pumpkin (Barth and Krause 1999). Subsequent experiments showed that PSI could be photoinhibited in chilling tolerant plants such as potato (Havaux and Davaud 1994), winter rye (Ivanov et al. 1998) and barley (Tjus et al. 1998). Barth and Krause (1999) compared the inhibition of PSI and PSII with cucumber, pumpkin, tobacco and spinach. At 4˚C, PSI was selectively photoinhibited in cucumber while both PSI and PSII were photoinhibited in other plant species. At 20˚C, both PSI and PSII were inhibited in cucumber while only PSII were inhibited in pumpkin, tobacco and spinach. Considering these past reports, it is apparent that 1) selective photoinhibition of PSI is observed at chilling temperature in certain chilling sensitive species, 2) PSI and PSII are similarly photoinhibited at chilling temperature in many other plant species, and 3) PSII is a main target of photoinhibition at room temperature.

Photoinhibition of PSII shows rather flat temperature dependence that can be explained by the effect through excitation pressure (i.e. reduction of plastoquinone pool or Q_A) (Huner et al. 1996), while photoinhibition of PSI in chilling sensitive plants shows threshold temperature below which the inhibition is induced (Terashima et al. 1994). The latter temperature dependence cannot be explained by the excitation pressure (Sonoike 1999). The difference in the temperature dependence of the PSI photoinhibition and PSII photoinhibition explains the fact that photoinhibition of PSI was mainly observed at chilling temperatures while that of PSII was observed at room temperatures (Sonoike 1998a). At chilling temperature, PSI photoinhibition was observed not only under experimental conditions but also in field grown plants (Teicher et al. 2000).

2. Protein degradation

Just as D1 protein is degraded upon photoinhibition of PSII, several subunits were reported to degrade upon photoinhibition of PSI. The main target of the protein degradation in PSI seems to be chlorophyll-binding reaction center subunits, PsaA/B (Sonoike and Terashima 1994). The degradation products of PsaB protein were identified by immunoblotting (Sonoike 1996b), and the sites of cleavage was further specified as on the loop exposed to the lumenal side between helices 7 and 8, and on the loop exposed to the stromal side between helices 8 and 9 (Sonoike et al. 1997). The latter cleavage site is near the ligands for the ion-sulfur center, F_X. Specific degradation of PsaB was also reported upon chilling in maize (Kingston-Smith and Foyer 2000), though there is no indication of the inhibition of PSI activity under such condition (Kingston-Smith et al. 1999).

Other subunits than PsaB were also reported to be degraded upon photoinhibition. Another reaction center subunit, PsaA, and small subunits exposed to the stromal side were reported to be degraded upon photoinhibition of PSI (Tjus et al.

1999). Photoinhibition of isolated PSI reaction center complexes induced by very high-intensity light caused a nonspecific degradation of PSI subunits (Baba et al. 1995). The degradation of PsaA/B proteins was also observed upon selective excitation of PSI with artificial electron donor to PSI (Tjus et al. 2001).

When cucumber leaves were treated at 4˚C for 5 h under the light at 190 μmol m^{-2} s^{-1}, less than 20% of the PsaA/B proteins were degraded just after the treatment (Fig. 1, lane 2). However, the PsaA/B proteins were gradually decreased in subsequent three days, and about 50% of the proteins were degraded on the third day after the treatment (Fig. 1, lane 3). From this result, we can conclude two points. First, the degradation of PsaA/B is not caused by the photo-cleavage during the inhibitory condition. Some enzymatic process must be involved in the degradation of PsaA/B. Secondly, the inhibited PSI complex are somehow 'tagged' during the photoinhibitory treatment, and the tagged complex is targeted by the proteolytic enzyme. Upon photoinhibition of PSI, iron-sulfur centers, F_A, F_B and F_X were destroyed (Sonoike et al. 1995a). Conformational change caused by the destruction of iron-sulfur centers may be the substance of the 'tag'. Some serine-type protease was suggested to be responsible for the degradation of PsaB protein (Sonoike et al. 1997). We propose that a serine-type protease degrades PsaA/B proteins whose conformation is changed by the destruction of iron-sulfur centers upon photoinhibition.

Fig. 1. Amount of PsaA/B protein per unit leaf area determined by Western blotting. 1: untreated, 2: chilled, 3: three days after the chilling treatment. The band intensity decreased to 85% and 52% in lane 2 and 3, respectively.

3. Recovery process after photoinhibition

Since the photoinhibition of PSI involves subunit degradation, and since PSI subunits are not rapid-turnover protein like D1 protein in PSII, the recovery rate of PSI from photoinhibition was predicted to be slow (Sonoike 1996a). Jung et al. (1998) observed that recovery of quantum yield of photosynthesis from chilling stress is not complete in two days while the recovery of Fv/Fm is complete in one day. The results imply that the slow recovery rate of PSI limits the recovery rate of overall photosynthesis after chilling stress. In fact, when we pursued recovery process of PSI activity after the chilling-induced photoinhibition of PSI by means of *in vitro* determination of P-700, the inhibition was still observed even after 6 days following the treatment (Fig. 2). Upon photoinhibition at chilling temperatures, underestimation of the PSI activity putatively due to the enhancement of cyclic electron flow around PSI was observed (Sonoike 1998b). Relatively fast recovery on the first day after the photoinhibitory treatment could be ascribed to the recovery of the rate of cyclic electron transfer. Although the recovery of PSI is very slow, the rate of the cyclic electron transfer returned to the normal level only in one day. As discussed

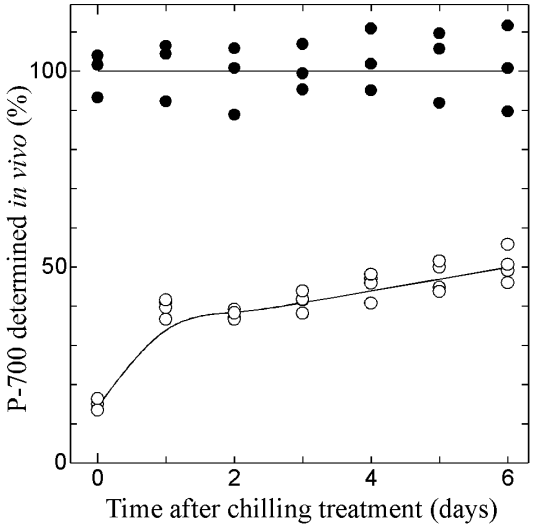

Fig. 2. Recovery process of the photooxidation of P-700 determined *in vivo*. Filled circles: untreated, open circles: chilled.

in the first section, both PSI and PSII are photoinhibited at low temperature regardless of the plant species. The recovery rate of PSII from photoinhibition is relatively fast so that PSI may become a limiting factor in photosynthesis in a few days. Even if PSI and PSII were similarly photoinhibited, the damage to plants in time range of a week would be mainly brought about by the photoinhibition of PSI.

4. Protective mechanisms in PSI

Since the damage to PSI is more dangerous than that to PSII, plants must have developed the protective mechanism in PSI. PSI is a major source of reactive oxygen species, so that the scavenging system for reactive oxygen species seems to be the primary protective mechanism in PSI. Terashima et al. (1998) demonstrated that *in vivo* concentration of hydrogen peroxide was temporarily increased under chilling condition. It was suggested that hydroxyl radical is a direct species that inactivates PSI (Sonoike 1996b). Fenton reaction between reduced iron-sulfur centers and hydrogen peroxide seems to trigger the photoinhibition of PSI (Sonoike 1996a). It should be noted that when oxidized form of P-700 is present in the complex, the reducing equivalent at the iron-sulfur centers could be safely scavenged through charge recombination. Thus, oxidized form of P-700 can be regarded as one of the protective mechanisms of PSI (Hihara and Sonoike 2001). Actually, Barth et al. (2001) proposed that the oxidized P-700 might protect PSI in several tropical plants under high light.

PSI was not photoinhibited in the absence of electron transfer from PSII (Sonoike 1995). Thus, another way to avoid the accumulation of reducing equivalent at the iron-sulfur centers is down-regulation of electron transfer itself. Failure of the down-regulation of electron transfer under stress conditions was reported to lead to cell death in *Chlamydomonas reinhardtii* (Wykoff et al. 1998) or in *Synechocystis* PCC 6803 (Sonoike et al. 2001). In cucumber leaves, chilling stress was reported to induce uncoupling of thylakoid membranes leading to the loss of proton gradient (Terashima et al. 1991). Thus, we suppose that down-regulation of electron transfer is cancelled during the chilling stress in cucumber leaves. To test whether the uncoupling of the thylakoid membranes were involved in the process of PSI photoinhibition, the effect of DCCD, an inhibitor of H^+-ATPase, was studied. It was revealed that DCCD had a partial protective effect on PSI (Table 1). The dark incubation of the DCCD-infiltrated leaves did not cause any inhibition (data not shown). Although DCCD is known to inhibit non-photochemical quenching as a side effect (Ruban et al. 1992), the inhibition of the dissipation of excess energy must lead to the enhancement of the inhibition of PSI, not to the protection of PSI. The loss of proton gradient in cucumber leaves under chilling condition may be the trigger of the photoinhibition of PSI under chilling stress. The infiltration of cucumber leaves with SF6847, an uncoupler, resulted in the decrease of photooxidizable P-700 determined *in vitro* by the weak light treatment even at room temperature (data not shown). Down-regulation of the electron transfer by proton

Table 1. Effects of DCCD on photochemical activities (expressed as % of untreated leaves)

	-DCCD	+DCCD
PSI[1]	22%	63%
PSII[2]	56%	67%

[1]P-700 determined in vitro. [2]Fv/Fm. Cucumber leaves were infiltrated with or without DCCD, ant then treated for 5 h at 4˚C under the light at 200 μmol $m^{-2}s^{-1}$.

gradient may be one of the main protective mechanisms in PSI.

Conclusion

In the past, the image of PSI was rather static compared with that of PSII. Now, it becomes clear that PSI also experiences inhibition and repair, and protective mechanisms are working inside and outside of the complex. The dynamic nature and regulatory aspects of PSI should be pursued in future.

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