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Different mechanisms for photosystem 2 reversible down-regulation in pumpkin and soybean leaves under saturating irradiance

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Abstract

After saturating irradiation for 3 h (SI), the original fluorescence F_0 increased while the photosystem 2 (PS2) photochemical efficiency (F_v/F_m) declined significantly. These parameters could largely recover to the levels of dark-adapted leaves after 3 h of subsequent dark recovery. No net loss of the D1 proteins occurred after SI. Soybean and pumpkin leaves had different responses to SI. Low temperature fluorescence parameters, F_{685} and F_{685}/F_{735}, decreased significantly in soybean leaves but not in pumpkin leaves. Part of the light-harvesting complex LHC2 dissociated from PS2 complexes in soybean leaves but not in pumpkin leaves, as shown by sucrose density gradient centrifugation and SDS-PAGE. The photon-saturated PS2 electron transport activity declined significantly in pumpkin thylakoids but not in soybean thylakoids. In addition, a large amount of phosphorylated D1 proteins was found in dark-adapted soybean leaves but not in dark-adapted pumpkin leaves. Hence at excessive irradiance soybean and pumpkin have the same protective strategy against photo-damage, reversible down-regulation of PS2, but two different mechanisms, namely the reversible down-regulation is related to the dissociation of LHC2 in soybean leaves but not in pumpkin leaves.

Additional key words: chlorophyll fluorescence; *Cucurbita*; D1 protein; dissociation; *Glycine*; light-harvesting complex 2 (LHC2); polyacrylamide gel electrophoresis; species differences; sucrose density gradient centrifugation.

Introduction

Under field conditions, photoinhibition of photosynthesis in soybean leaf may be attributed to photosystem 2 (PS2) reversible down-regulation rather than photo-damage, especially the net loss of the D1 proteins (Xu and Wu 1996, Hong and Xu 1999a). Also the reversible down-regulation in soybean leaf is related to the dissociation of LHC2 from PS2 complex (Hong and Xu 1999b). However, the mechanism for energy dissi-pation by the dissociated LHC2 remains unclear.

As a core component, the D1 protein plays a key role in the structure and function of PS2 complex and has a rapid turnover rate (Barber and Andersson 1992, Critchley and Russell 1994, Mulo *et al.* 1997). Under normal conditions, the degradation and synthesis of the D1 protein *in vivo* are co-ordinated and no net loss of the D1 proteins is observed in higher plants. If its rate of synthesis is slower than that of its degradation under severe stress, such as high irradiance and low temperature, the damage to PS2 complex will occur due to a net loss of the D1 proteins (Ohad *et al.* 1990). Phosphorylation of the D1 protein may regulate the turnover of the D1 proteins. The phosphorylation of the D1 protein retards the degradation of damaged D1 protein under stress conditions when rapid replacement by a new D1 copy is not possible (Rintamäki *et al*. 1995). So phosphorylation of the D1 protein could protect PS2 complex from total disassembly and degradation of the PS2 subunits (Ebbert and Godde 1996, Kruse *et al.* 1997, Baena-Gonzalez *et al.* 1999).

In a study on the relationship between D1 protein

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Abbreviations: Chl, chlorophyll; DA, dark-adapted leaves; DM, *n*-dodecyl-β-D-maloside; DR, dark-recovered leaves; F_m, maximum Chl fluorescence yield when all PS2 are closed; F_0, initial Chl fluorescence yield when all PS2 are open; F_v/F_m, potential photochemical efficiency of PS2; F_{685}, Chl fluorescence yield from PS2 antenna measured at 77 K; F_{735}, Chl fluorescence yield from PS1 antenna measured at 77 K; LHC2, Chl *a/b* light-harvesting complex 2; PMSF, phenylmethane-sulfonyl fluoride; PPFD, photosynthetic photon flux density; PS, photosystem; SI, saturating irradiation; 1,4-BQ, 1,4-benzoquinone.
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phosphorylation and reversible down-regulation of PS2, we found a substantial amount of phosphorylated D1 proteins ($D1^*$) in dark-adapted soybean leaf (Zhang *et al.* 2002). This is different from the results that no $D1^*$ was observed in the dark-adapted pumpkin leaves (Kettunen *et al.* 1991). The difference in D1 protein phosphorylation level in dark-adapted leaves may imply a difference in protective mechanisms against photodamage between pumpkin and soybean leaves.

In order to examine the validity of the supposition, a comparative study on the response to saturating irradiation (SI) between pumpkin and soybean leaves was made by chlorophyll (Chl) fluorescence analysis, SDS-PAGE, Western blotting, sucrose density gradient centrifugation, and measurement of photosynthetic electron transport activity.

Materials and methods

Plants: Pumpkin (*Cucurbita pepo* L.) and soybean (*Glycine max* L.) plants were grown in pots under field conditions. The plants were irrigated every day to avoid water stress. The third or fourth (counted from the top of plant) fully expanded, healthy leaves (with a F_v/F_m above 0.82) were used in experiments.

Saturating irradiance: Pumpkin and soybean plants grown in pots were transferred from the field to the laboratory. Following dark-adaptation for 14 h (over-night), pumpkin and soybean leaves were irradiated for 3 h at a PPFD of 1 000 μmol m^{-2} s^{-1} (the saturating irradiance for leaf photosynthesis of pumpkin and soybean grown in the field). To remove heat, radiation from a halogen lamp (1 000 W) was allowed to pass through a layer of flowing water between the lamp and the leaves.

Chl fluorescence analysis: The initial Chl fluores-cence (F_0) and photosystem 2 (PS2) photochemical effici-ency (F_v/F_m) in soybean and pumpkin leaves at room temperature were measured with a portable Chl fluorometer, *PAM-2000* (*Walz*, Effeltrich, Germany) as described by Hong and Xu (1999b). Low-temperature Chl fluorescence was measured at 77 K with a 44 W-fluorescence spectrofluorimeter as described by Hong and Xu (1999b).

Sodium dodecyl sulfate (SDS)-PAGE and immunoblotting: Callahan *et al.* (1990) found that phosphorylated D1 protein ($D1^*$) could be separated from non-phosphorylated one (D1) by its slightly slower mobility in SDS-PAGE. This method is convenient and effective to identify the two forms of the D1 protein and was used in the present study. Thylakoids were isolated from soybean and pumpkin leaves as described by Hong and Xu (1999a). All solutions used in the isolation process contained 1 mM PMSF (phenylmethanesulfonyl fluoride, *Merck*) to inhibit protease activity. Thylakoid membrane proteins were resolved by SDS-PAGE using the system of Laemmli (1970) with a 15 % resolving gel and a 5 % stacking gel, both containing 6 M urea. For the separation of $D1^*$ and D1, SDS-PAGE was carried out on 16×18×0.75 cm slabs of *SE600* system (*Pharmacia*) until a 29 kDa marker was about 2 cm from the bottom of the resolving gel.

For the quantification of the D1 protein, SDS-PAGE was performed on 10×8×0.75 cm slabs of Mini-Protein 3 Cell system (*Bio-Rad*).

By Western blotting analysis, the D1 protein was identified using a monoclonal anti-D1 antibody (a kind gift from Prof. Jian-Ren Shen). SDS-PAGE resolved polypeptides were electrophoretically transferred to Hybond^{TM} ECL^{TM} nitrocellulose membrane (*Pharmacia*) with a semi-dry transfer cell (*Pharmacia*). The D1 protein was immunodetected using the ECL assay kit (*Pharmacia*). For quantification of the D1 protein, the immunoblots were scanned with a laser densitometer (*Gel-Doc*).

Sucrose density gradient centrifugation was carried out according to Hong and Xu (1999b) with some modifications. The thylakoid membrane preparation with Chl content of 2 kg m^{-3} was mixed with the same volume of 2 % *n*-dodecyl-β-D-maloside (β-DM) and incubated at 4 °C for 10 min. Then 1 cm^3 of the mixture was loaded onto a 0.1-1.0 M liner sucrose gradient in a medium containing 10 mM Tricine (pH 7.8), 10 mM NaF, and 0.03 % β-DM. The samples were spun in a *Beckman SW41* rotor at 650 rps and 4 °C for 16 h. Individual green layers were harvested with a syringe and stored at –20 °C.

Measurement of PS2 electron transport activity of isolated thylakoids was performed according to Zhang *et al.* (1988) with some modification. The activity was measured by a Clark-type electrode (assembled by the Shanghai Institute of Plant Physiology) using 0.5 mM 1,4-benzoquinone as electron acceptor. The final Chl amount was 0.2 mg in the reaction mixture. Considering the difference among repeated experiments, the electron transport activity was given as percent of control in this study.

Results

Changes in Chl fluorescence parameters after SI and subsequent dark recovery: As shown by Table 1, the SI of 1 000 μmol m^{-2} s^{-1} for 3 h led to a significant elevation in F_0 and decline in F_v/F_m for both pumpkin and soybean leaves compared with those of dark-adapted control. Also, after 3 h of subsequent dark recovery, both F_0 and F_v/F_m could largely recover to the levels of dark-adapted leaves, indicating that the changes in F_0 and F_v/F_m caused by SI are reversible.

Changes in amount of D1 protein after SI and subsequent dark recovery: A linear relationship of the Western blotting reaction was observed between the amounts of the D1 protein and the thylakoid membrane samples loaded onto the gel when the Chl amount of the sample was between 5.0–12.5 μg (Zhang *et al.* 2002). Hence the Western blotting can be used in relative quantification of the D1 protein. After SI and subsequent dark recovery, the D1 protein of soybean and pumpkin leaves remained roughly the same as that of dark-adapted leaves (Fig. 1), indicating that the SI does not cause the net loss of D1 proteins in these leaves.

Table 1. Changes in the chlorophyll fluorescence parameters of soybean and pumpkin leaves after saturating irradiation and subsequent dark recovery. Means of 8-10 leaves with standard error (±), and the values in parentheses are the percentage of dark-adapted leaves (DA). Asterisk (*) indicates that the difference between saturating-irradiated and dark-adapted leaves is significant (p<0.05). DA – dark-adapted leaves; SI – saturating-irradiated leaves; DR – dark-recovered leaves.

Species	Treatment	F_0	F_v/F_m
Soybean	DA	0.159 ± 0.003 (100)	0.841 ± 0.003 (100)
	SI	0.192 ± 0.003* (121)	0.698 ± 0.002* (83)
	DR	0.166 ± 0.002 (104)	0.818 ± 0.002 (97)
Pumpkin	DA	0.130 ± 0.005 (100)	0.832 ± 0.024 (100)
	SI	0.170 ± 0.009* (131)	0.700 ± 0.014* (84)
	DR	0.135 ± 0.006 (104)	0.798 ± 0.003 (96)

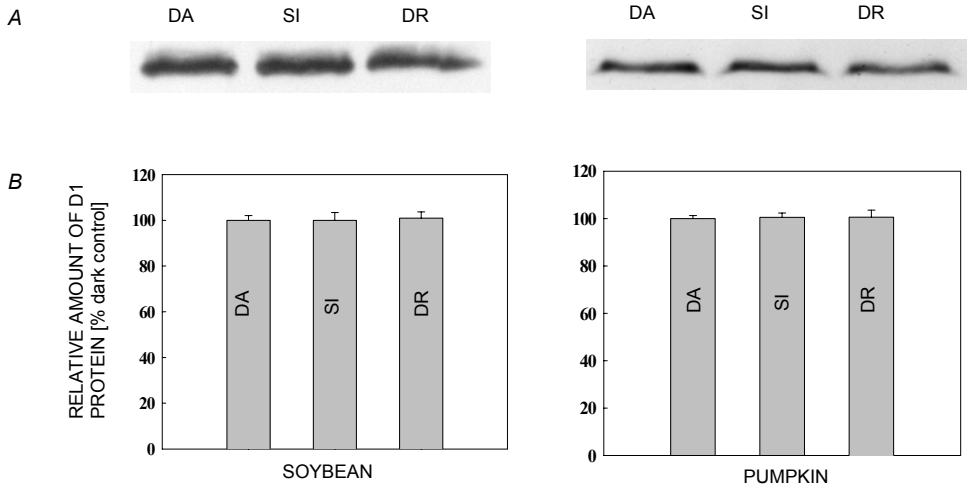

Fig. 1. Changes in the D1 protein amount of soybean and pumpkin leaves after saturating irradiation (SI) and subsequent dark recovery (DR). (*A*) Western blotting; (*B*) quantitative demonstration of the D1 proteins by a *Gel-Doc* laser densitometer. Means of 3-4 repeats with the standard error.

Effect of SI on low tempera-ture fluorescence parameters: The F_{685} (the fluores-cence yield from PS2 antenna) declined significantly in soybean leaves (Fig. 2*A*) but not in pumpkin leaves (Fig. 2*B*) after SI. Hence SI leads to the dissociation of some LHC2s from PS2 complex in the former but not in the latter. Nevertheless, no significant change in F735 (the fluores-cence yield from PS1 antenna) was observed in soybean leaves after SI for 3 h, implying that the dissociated LHC2 does not migrate to PS1.

Effect of SI on LHC2 dissocia-tion: In general, four green layers were observed after sucrose density gradient centrifugation of thylakoid membrane samples. Layer *4*

contains mainly the PS1-LHC1 complexes, and layer *3* contains the PS2-LHC2 super-complex. Layers *2* and *1* usually consist of major and minor LHC2 pigment protein complexes (Barbato *et al.* 1992). In our experiment, the green colour in layer *3* became lighter, while the green colour in layers *1* and *2* became darker for thylakoid samples from SI treated soybean leaves as compared with those for thylakoid samples from dark-adapted leaves (Fig. 3*A*). SDS-PAGE of these layers showed that the relative amount of LHC2s decreased in layer *3* and increased in layers *1* and *2* (Fig. 3*C*) for thylakoid samples from SI soybean leaves, as compared with those for thylakoid samples from the dark-adapted leaves. Hence after SI a part of LHC2s dissociates from the PS2-LHC2 super-complex in soybean leaves. However, no such changes were observed for thylakoid samples from SI pumpkin leaves (Fig. 3*B*,*D*), implying that no dissociation of LHC2 from PS2 complex occurs in pumpkin leaves.

Changes in the PS2 electron transport activity of thylakoids from SI leaves: The PS2 electron transport activity measured at SI had no change in thylakoids from the SI soybean leaves, compared with those from dark control leaves, but a significant decrease in the activity (to 81 % of the control) was observed when measured at weak irradiance. However, even if measured at SI a significant decline (to 77 % of the control) in the PS2 electron transport activity was observed in the thylakoids from the SI pumpkin leaves (Table 2).

Table 2. Effects of saturating irradiation on the photosynthetic electron transport activity of photosystem 2, PS2 ($H_2O \rightarrow$ 1,4-BQ) in soybean and pumpkin thylakoids. Percent values in relation to dark control. The photosynthetic electron transport activities of PS2 ($H_2O \rightarrow$ 1,4-BQ) of dark control thylakoids were 62.5–91.7 and 31.9–41.7 mmol(O_2) kg^{-1}(Chl) s^{-1} measured at saturating and weak irradiance, respectively. * and ** indicate significant levels of differences between thylakoids from saturating-irradiated leaves and those from dark control leaves for $p<0.05$ and $p<0.01$, respectively.

Species	Measuring irradiance	Repeat 1	2	3	4	Mean
Soybean	saturating	95.6	106.7	99.6	103.8	101.4 ± 2.4
	weak	80.9	82.7	81.2	79.7	81.1 ± 0.6*
Pumpkin	saturating	80.5	78.5	81.2	69.1	77.3 ± 2.8**

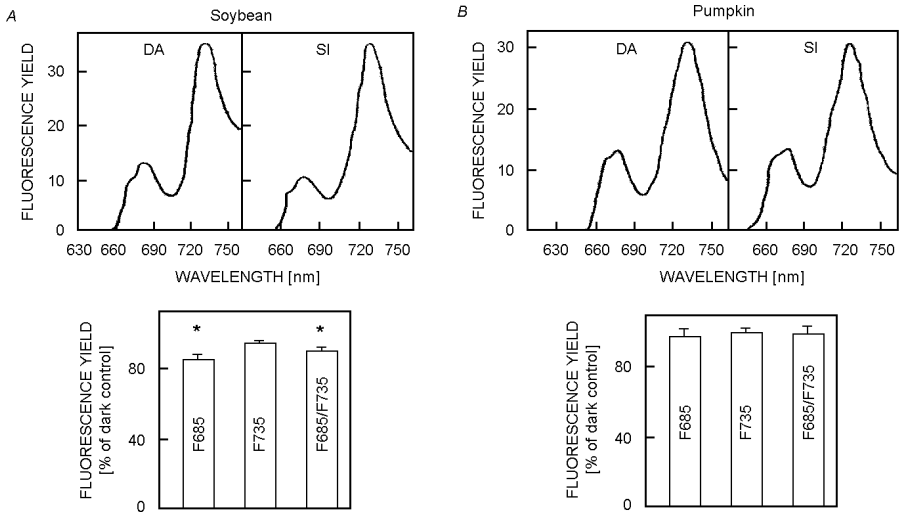

Fig. 2. Effect of saturating irradiation (SI) on the low-temperature fluorescence in soybean (*A*) and pumpkin (*B*) leaf. Fluorescence emission spectra in the statistical 8 repeats recorded by a fluorescence spectrofluorimeter at 77 K. Qualitative demonstrations of the fluorescence parameters of F_{685}, F_{735}, and F_{685}/F_{735} (*top*) and their quantitative evaluations (*bottom*): each column represents the mean of 8 repeats with standard error. * indicates that the difference between saturating-irradiated and dark-adapted leaves is significant ($p<0.05$).

Phosphorylation level of D1 proteins in soybean and pumpkin leaves: Immunoblots of thylakoid membrane proteins from soybean and pumpkin leaves showed two forms of the D1 protein, a phosphorylated (D1*) and a non-phosphorylated (D1) one (Fig. 4*A*). Fig. 4*B* and 4*C* are qualitative demonstrations of D1* and D1 in the immunoblots (Fig. 4*A*). As shown in Fig. 4, the percentage of D1* could be up to 41 % of total D1 proteins in fully dark-adapted soybean leaves. However, no D1* was found in fully dark-adapted pumpkin leaves. This is consistent with the results of Kettunen *et al*. (1991). After SI, the percentages of D1* increased to 58 and 56 %, and

after dark recovery for 3 h the values declined to 47 and 49 % of total D1 proteins, respectively, for soybean and pumpkin leaves, showing that part of D1* could be dephosphorylated in the dark.

Discussion

The same protective strategy against photodamage in soybean and pumpkin leaves: Similar responses of pumpkin and soybean leaves to SI, reversible increase in F_0, and decrease in F_v/F_m reported here (Table 1) imply that the two species may have the same protective strategy against photo-damage of the photosynthetic apparatus at excess irradiance.

The increase in F_0 has been taken as a sign of damage of PS2 reaction centres, mainly due to a net loss of the D1 proteins (Franklin *et al.* 1992) or down-regulation of PS2 (Krause and Weis 1991, Critchley and Russell 1994, Yamane *et al.* 1997). In this study, the increase in F_0 of both soybean and pumpkin leaves is mainly due to the down-regulation of PS2 rather than its damage and the down-regulation is reversible. The conclusions are based on the following facts. First, after 3 h in the dark both F_0 and F_v/F_m changed by saturating irradiation, could largely recover to the levels in dark-adapted leaves (Table 1). Second, no net loss of the D1 proteins was observed after SI (Fig. 1). Third, after the recovery of F_0 and F_v/F_m in the dark, the D1 protein amount remained similar as that of SI leaves (Fig. 1), indicating that the recovery of PS2 function is not likely related to the D1 protein synthesis (Hong and Xu 1999a). Such reversible PS2 photoinactivation without net loss of the D1 protein was reported in spinach thylakoids from photoinhibited leaves (Schnettger *et al.* 1992).

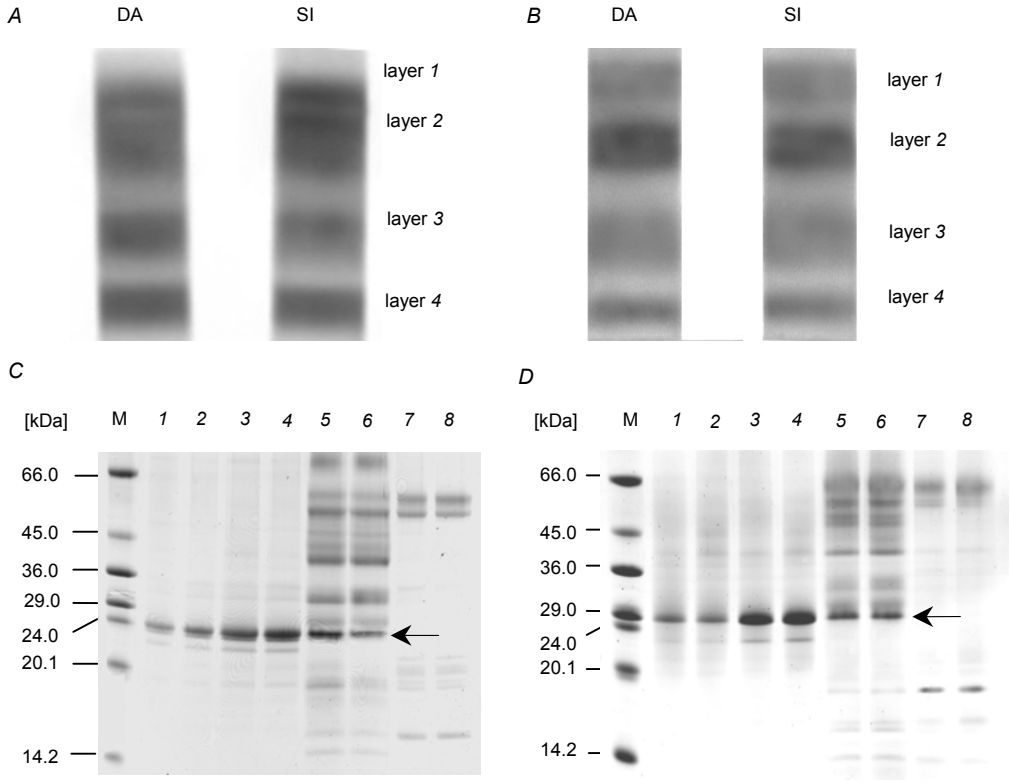

Fig. 3. Sucrose density gradient centrifugation of soybean (*A*) and pumpkin (*B*) thylakoid samples. DA – thylakoids from dark-adapted leaves; SI – thylakoids from saturating-irradiated leaves. *C*, *D* – SDS-PAGE analysis of the thylakoid compositions from different layers in (*A*) and (*B*), respectively. M – standard protein marker. Lanes *1*, *3*, *5*, and *7* for layers *1*, *2*, *3*, and *4* of DA, and lanes *2*, *4*, *6*, and *8* for layers *1*, *2*, *3*, and *4* of SI, respectively. The arrows show LHC2.

Two different mechanisms of PS2 down-regulation in soybean and pumpkin leaves: Although pumpkin and soybean leaves have the same protective strategy, the reversible down-regulation of PS2 and their mechanisms may differ from each other. The down-regulation is related to the dissociation of part of the LHC2 from PS2 complex in soybean leaves but not in pumpkin leaves, as shown by the Chl fluorescence analysis at low temperature and sucrose density gradient centrifugation.

At low temperature (77 K) the Chl fluorescence emission peak at 685 nm (F_{685}) stems from the core antenna of PS2 (Siefermann-Harms 1988, Bassi *et al.* 1990, Krause

and Weis 1991). However, the peripheral antenna LHC2 also contributes to F_{685} because photons absorbed by LHC2 can be transferred to the core antenna providing that the LHC2 is associated with PS2 core complex. A change in F_{685}, therefore, can reflect the change in status of association of LHC2 and PS2 core complex. Namely, a decrease in F_{685} indicates the dissociation of some LHC2s from PS2 core complexes, while an increase in F_{685} indicates the re-association of the dissociated LHC2s with PS2 core complexes. So the significant decrease in F_{685} in soybean leaves (Fig. 2*A*) indicates a reduction of LHC2 amount associated with PS2 core complexes. For the reduction there are two possible reasons: the dissociation of some LHC2s from PS2 complexes and the damage of some LHC2s. Nevertheless, the possibility of the damage of some LHC2s can be ruled out by the rapid recovery of F_{685} and F_{685}/F_{735} in the dark (Hong and Xu 1999b, Cai and Xu 2002). So the dissociation of some LHC2s from the PS2 complex is the sole reason for the reduction of LHC2 amount. Such dissociation of some LHC2s can protect PS2 reaction centres against photo-damage by the decrease in the size of antenna of PS2 complex and thereby the amount of excitation energy transferred to PS2 complex. Since PS2 core antenna rather than LHC2 emits F_{685}, the dissociation of some LHC2s can lead to a decrease but not a complete loss or quenching of F_{685}. In contrast with soybean leaves, no such decrease in F_{685} (Fig. 2*B*) was observed in pumpkin leaves after SI, indicating that no dissociation of LHC2 from PS2 complex takes place.

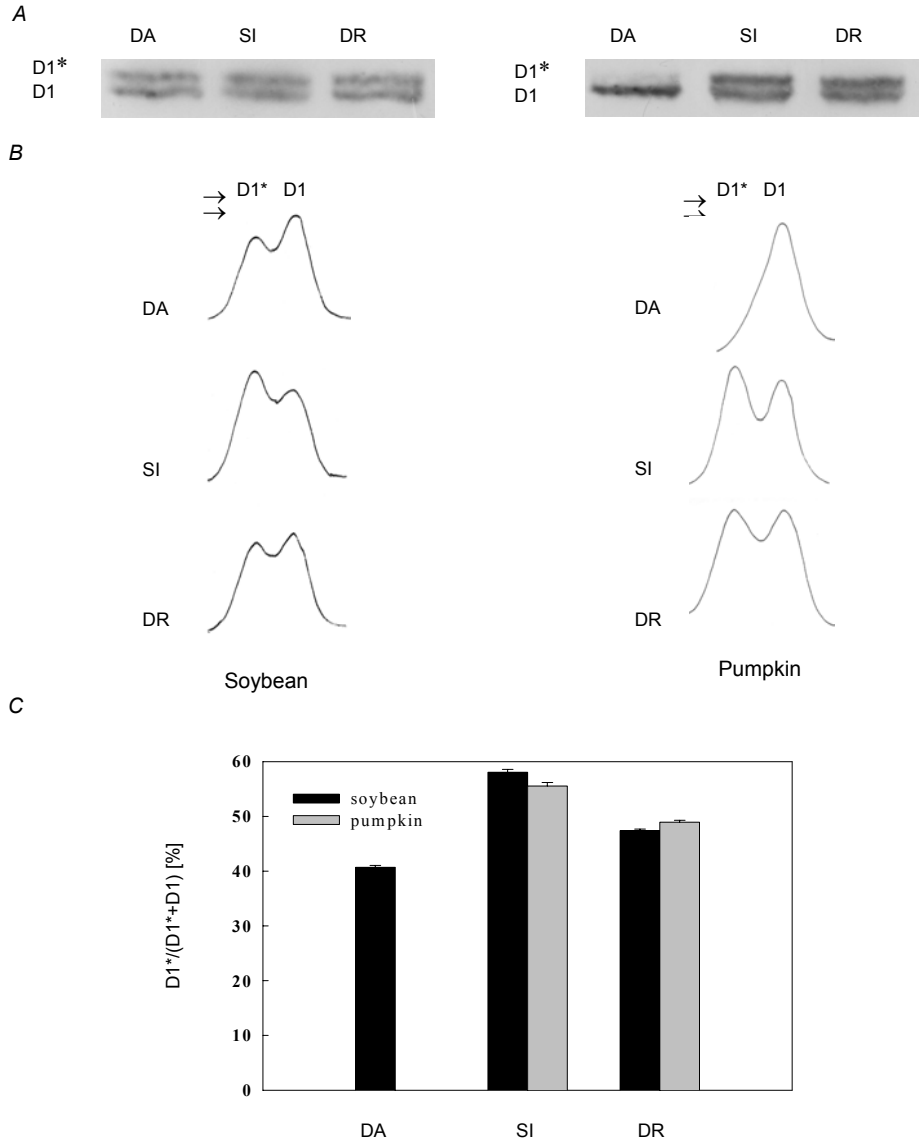

Fig. 4. Qualitative demonstration of non-phosphorylated and phosphorylated D1 protein (D1 and D1*) in dark-adapted (DA), saturating-irradiated (SI), and subsequently dark-recovered (DR) leaves of soybean and pumpkin. (*A*) Western blotting; (*B*) laser densitograms of D1 and D1*; (*C*) qualitative demonstration of D1*/(D1+D1*) [%]. Means of 3-4 repeats with standard error.

The sucrose density gradient centrifugation and the following SDS-PAGE (Fig. 3) also support this deduction about LHC2 dissociation. Following sucrose density gradient centrifugation, the green colour of the layer *3* became lighter while the colour of the layers *1* and *2* became darker for the thylakoid preparations from SI soybean leaves (Fig. 3*A*). Also, the SDS-PAGE indicated that the relative amount of LHC2s decreased in the layer *3* while it increased in the layers *1* and *2* when a similar volume of samples from each layer was added into the gel (Fig. 3*C*). However, for thylakoid preparation from SI pumpkin leaves no such changes were observed (Fig. 3*B*, *D*).

Surprisingly, besides the obvious difference in the LHC2 dissociation after SI, there was also a substantial difference in the phosphorylation level of the D1 proteins of the dark-adapted leaves between pumpkin and soybean leaves. In the fully dark-adapted soybean leaves there was a high amount of phosphorylated D1 proteins, but no phosphorylated D1 protein was found in the fully dark-adapted pumpkin leaves, in which no dissociation of LHC2 occurred after SI (Fig. 4). It is possible that the accumulation of the phosphorylated D1 proteins in the dark is a prerequisite for the dissociation of LHC2 from PS2 complex in soybean leaves. Phosphorylation of the D1 protein is important in protecting the photosynthetic apparatus, especially the PS2 reaction centres, against photo-damage caused by intense irradiance (Koivuniemi *et al.* 1995, Rintamäki *et al.* 1995). However, the relationship between the phosphorylated D1 proteins and the dissociation of LHC2 is worth further exploring.

The different mechanisms of the reversible down-regulation of PS2 for soybean and pumpkin leaves may be a reflection of the bio-diversity in protecting the photosynthetic apparatus from photo-damage under stress.

Apparent and actual inactivation of PS2 reaction centres: The reversible inactivation of PS2 reaction centres in soybean leaves reported previously (Xu and Wu 1996, Hong and Xu 1999a) may be an apparent inactivation. Namely, it is mainly due to the dissociation of LHC2 from PS2 complex rather than the loss of the function of PS2 reaction centre itself. The following facts support this deduction: (*1*) There was no net loss of the D1 proteins after SI (Fig. 1). The constancy of the D1 protein amount provides the essential prerequisite for the normal function of PS2 reaction centres. (*2*) The electron transport activity of PS2 was not changed in the thylakoids from SI soybean leaves when measured at SI, but it was significantly declined when measured at weak irradiance (Table 2). Dissociation of part of LHC2s can decrease both the photon absorption cross-section and the excitation energy transferred to the PS2 reaction centres, resulting in a decrease in PS2 photochemical efficiency, expressed as F_v/F_m (Table 1). When irradiance is limited, a smaller light-harvesting antenna absorbs and transfers less photons to PS2 reaction centre, leading to a decrease in the charge separation rate of the centre, and thereby to a substantial decline in the electron transport activity of PS2. However, it can not induce the decline in the PS2 electron transport rate measured at the SI because the excessive photons can compensate the reduction in light-harvesting cross-section.

The apparent inactivation or down-regulation of PS2, caused by the dissociation of LHC2, can decrease the effective light-harvesting antenna size of the PS2 reaction centres and thereby reduce the pressure of excitation energy on PS2 at high irradiance. Therefore, it may be an effective protective strategy against photo-damage to the photosynthetic apparatus (Zhang and Xu, unpublished). How the dissociated LHC2 dissipates the absorbed excitation energy as heat is worth studying.

Although the reversible down-regulation of PS2 in soybean leaves involves both the dissociation of LHC2 from PS2 and the change in the light-harvesting cross section of PS2 complex, it is different from state transition (Tan *et al.* 1998, Hong and Xu 1999b).

Different from the apparent inactivation of PS2 reaction centres that occurs in soybean leaves, the inactivation without the dissociation of LHC2 in pumpkin leaves is an actual one, because we observed a significant loss of PS2 function, shown by the significant decline in the light-saturated PS2 electron transport activity (Table 2). Some changes can lead to the decline in the PS2 electron transport activity. The conformational changes of the D1 protein can cause the functional loss of PS2 (Ohad *et al.* 1990). Also the de-stabilisation of the secondary electron acceptor Q_B^- can lead to an impairment of the PS2 electron transport activity (Giardi *et al.* 1992, Lavergne and Briantais 1996). Alternatively, the inhibition of the PS2 electron transport activity may also be caused by the accumulation of reduced Q_A after irradiation (Vass *et al.* 1992). It is not sure whether these changes occur or not during the down-regulation of PS2 in pumpkin leaves. Thus the mechanism for reversible inactivation of PS2 reaction centres in pumpkin leaves should be further studied.

References

Baena-Gonzalez, E., Barbato, R., Aro, E.-M.: Role of phosphorylation in the repair cycle and oligomeric structure of PSII. – Planta **208**: 196-204, 1999.

Barbato, R., Friso, G., Rigoni, F., dalla Vecchia, F., Giacometti, G.M.: Structure changes and lateral redistribution of photosystem II during donor side photoinhibition of thylakoids. – J. Cell Biol. **119**: 325-335, 1992.

Barber, J., Andersson, B.: Too much of a good thing: light can be bad for photosynthesis. – Trends biochem. Sci. **17**: 61-66, 1992.

Bassi, R.L., Rigoni, F., Giacometti, G.M.: Chlorophyll binding proteins with antenna function in higher plants and green algae. – Photochem. Photobiol. **52**: 1187-1206, 1990.

Cai, S.-Q., Xu, D.-Q.: Light intensity-dependent reversible down-regulation and irreversible damage of PSII in soybean leaves. – Plant Sci. **163**: 847-853, 2002.

Callahan, F.E., Ghirardi, M.L., Sopory, S.K., Mehta, A.M., Edelman, M., Mattoo, A.K.: A novel metabolic form of the 32 kDa-D1 protein in the grana-localized reaction center of photosystem II. – J. biol. Chem. **265**: 15357-15360, 1990.

Critchley, C., Russell, A.W.: Photoinhibition of photosynthesis *in vivo*: The role of protein turnover in photosystem II. – Physiol. Plant. **92**: 188-196, 1994.

Ebbert, V., Godde, D.: Phosphorylation of PS II polypeptides inhibits D1 protein-degradation and increases PS II stability. – Photosynth. Res. **50**: 257-269, 1996.

Franklin, L.A., Levavasseur, G., Osmond, C.B., Henley, W.J., Ramus, J.: Two components of onset and recovery during photoinhibition of *Ulva rotundata*. – Planta **186**: 309-408, 1992.

Giardi, M.T., Rigoni, F., Barbato, R.: Photosystem II core phosphorylation heterogeneity, differential herbicide binding, and regulation of electron transfer in photosystem II preparations from spinach. – Plant Physiol. **100**: 1948-1954, 1992.

Hong, S.-S., Xu, D.-Q.: Light-induced increase in initial chlorophyll fluorescence F_0 level and the reversible inactivation of PS II reaction centers in soybean leaves. – Photosynth. Res. **61**: 269-280, 1999a.

Hong, S.-S., Xu, D.-Q.: Reversible inactivation of PSII reaction centers and the dissociation of LHCII from PSII complex in soybean leaves. – Plant Sci. **147**: 111-118, 1999b.

Kettunen, R., Tyystjärvi, E., Aro, E.-M.: D1 protein degradation during photoinhibition of intact leaves. A modification of the D1 protein precedes degradation. – FEBS Lett. **290**: 153-156, 1991.

Koivuniemi, A., Aro, E.-M., Andersson, B.: Degradation of D1- and D2-proteins of photosystem II in higher plants is regulated by reversible phosphorylation. – Biochemistry **34**: 16022-16029, 1995.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Kruse, O., Zheleva, D., Barber, J.: Stabilization of photosystem II dimers by phosphorylation: implication for the regulation of the turn of D1 protein. – FEBS Lett. **48**: 276-280, 1997.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. – Nature **227**: 680-685, 1970.

Lavergne, J., Briantais, J.-M.: Photosystem II hererogeneity. – In: Ort, D.R., Yocum, C.F. (ed.): Oxygenic Photosynthesis: The Light Reactions. Pp. 265-287. Kluwer Academic Publishers, Dordrecht – Boston – London 1996.

Mulo, P., Tyystjärvi, E., Govindjee, Maenpaa, P., Aro, E.-M.: Mutagenesis of the D-E loop of photosystem II reaction center protein D1. Function and assembly of photosystem II. – Plant mol. Biol. **33**: 1059-1071, 1997.

Ohad, I., Adir, N., Koike, H., Kyle, D.J., Inoue, Y.: Mechanism of photoinhibition *in vivo*. A reversible light-induced conformational change of reaction center II is related to an irreversible modification of the D1 protein. – J. biol. Chem. **265**: 1972-1979, 1990.

Rintamäki, E., Kettunen, R., Tyystjärvi, E., Aro, E.-M.: Light-dependent phosphorylation of D1 reaction centre protein of photosystem II: hypothesis for the functional role in vivo. – Physiol. Plant. **93**: 191-195, 1995.

Schnettger, B., Leitsch, J., Krause, G.H.: Photoinhibition of photosystem 2 *in vivo* occurring without net D1 protein degradation. – Photosynthetica **27**: 261-265, 1992.

Siefermann-Harms, D.: Fluorescence properities of isolated chlorophyll-protein complexes. – In: Lichtenthaler, H.K. (ed.): Applications of Chlorophyll Fluorescence. Pp. 45-54. Kluwer Academic Publishers, Dordrecht – Boston – London 1988.

Tan, X.-X., Xu, D.-Q., Shen, Y.-K.: Both spillover and light absorption cross-section changes are involved in the regulation of excitation energy distribution between the two photosystems during state transitions in wheat leaf. – Photosynth. Res. **56**: 95-102, 1998.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.-M., Andersson, B.: Reversible and irreversible intermediates during photoinhibition of photosystem II: Stable reduced Q_A species promote chlorophyll triplet formation. – Proc. nat. Acad. Sci. USA **89**: 1408-1412, 1992.

Xu, D.-Q., Wu, S.: Three phases of dark-recovery course from photoinhibition resolved by the chlorophyll fluorescence analysis in soybean leaves under field conditions. – Photosynthetica **32**: 417-423, 1996.

Yamane, Y., Kashino, Y., Koike, H., Satoh, K.: Increase in the fluorescence F_0 level and reversible inhibition of photosystem II reaction center by high-temperature treatments in higher plants. – Photosynth. Res. **52**: 57-64, 1997.

Zhang, H.-B., Cai, S.-Q., Xu, D.-Q.: D1 protein phosphorylation/dephosphorylation alone has no effect on the electron transport activity of photosystem II in soybean leaves. – Plant Sci. **162**: 507-511, 2002.

Zhang, Q.-D., Zhang, S.-P., Zhang, Q.-F.: [Photosynthetic functions of rice improved by 2,3-epoxypropionate.] – Acta bot. sin. **30**: 54-61, 1988. [In Chin.]