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Role of light-harvesting complex 2 dissociation in protecting the photosystem 2 reaction centres against photodamage in soybean leaves and thylakoids

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Abstract

The protective role of light-harvesting complex 2 (LHC2) dissociation from photosystem 2 (PS2) complex was explored by the 5'-*p*-fluorosulfonylbenzoyl adenosine (FSBA, an inhibitor of protein kinase) treatment at saturating irradiance (SI) in soybean leaves and thylakoids. The dissociation of some LHC2s from PS2 complex occurred after SI treatment, but FSBA treatment inhibited the dissociation as demonstrated by analysis of sucrose density gradient centrifugation of thylakoid preparation and low-temperature (77 K) chlorophyll (Chl) fluorescence. A significant increase in F_0 and decrease in F_v/F_m occurred after SI, and the two parameters could largely recover to the levels of dark-adapted leaves after subsequent 3 h in the dark, but they could not recover in the FSBA-treated leaves at SI. Neither the electron transport activity of PS2 nor the D1 protein amount *in vivo* had significant change after SI without FSBA, whereas FSBA treatment at SI could result in significant decreases in both the PS2 electron transport activity and the D1 protein amount. When thylakoids instead of leaves were used, the PS2 electron transport activity and the D1 protein amount declined more after SI with FSBA than without FSBA. The phosphorylation level of PS2 core proteins increased, while the phosphorylation level of LHC2 proteins was reduced after SI. Also, the phosphorylation of PS2 core proteins could be greatly inhibited by the FSBA treatment at SI. Hence in soybean leaf the LHC2 dissociation is an effective strategy protecting PS2 reaction centres against over-excitation and photodamage by reducing the amount of photons transferred to the centres under SI, and the phosphorylation of PS2 core proteins plays an important role in the dissociation.

Additional key words: chlorophyll fluorescence; D1 protein; 5'-*p*-fluorosulfonylbenzoyl adenosine; *Glycine*; protein phosphorylation; PS2 reaction centre.

Introduction

In the irradiance fluctuating environment, plants have evolved some strategies to maximise photon harvesting at low irradiance and reduce photon absorption at high irradiance for optimisation of photosynthesis, growth, and development. Plants can adjust the composition and structure of the photosynthetic apparatus to acclimate to long-term changes in radiation quality and irradiance (Anderson 1986, Smith *et al.* 1990, Walters and Horton 1994). In response to short-term radiation fluctuations, plants can alter the absorption and utilisation of photon energy by the movement of leaf and/or chloroplast (Haupt and Scheuerlein 1990, Björkman and Demmig-Adams 1994, Kagawa *et al.* 2001), state transition (Fork and Satoh 1986), and thermal dissipation (Demmig *et al.* 1987, Niyogi 1999, Xu and Shen 1999).

There are several mechanisms for thermal dissipation of excitation energy in plants. Among them the xanthophyll cycle-dependent one has been intensely investigated

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Abbreviations: 1,4-BQ, 1,4-benzoquinone; Chl, chlorophyll; DM, *n*-dodecyl-β-D-maltoside; F_m, maximum chlorophyll fluorescence yield when all PS2 are closed; F_0, initial chlorophyll fluorescence yield when all PS2 are open; FSBA, 5'-*p*-fluorosulfonylbenzoyl adenosine; F_v/F_m, photochemical efficiency of PS2 in dark-adapted leaf; LHC2, chlorophyll *a/b* light-harvesting complex 2; PMSF, phenylmethane-sulfonyl fluoride; PPFD, photosynthetic photon flux density; PS2, photosystem 2; RC, reaction centre; SI, saturating irradiance; TD, thylakoids from dark-adapted leaves; TF, thylakoids from FSBA-treated leaves at SI; TL, thylakoids from SI-leaves.
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(Demmig-Adams and Adams 1996, Niyogi *et al.* 1997, Niyogi 1999), while the reaction centre (RC) inactivation-dependent mechanism has been studied only rarely. The inactivated PS2 RCs can dissipate excessive excitation energy and thereby protect the neighbouring functional PS2 RC from photodamage (Critchley and Russell 1994, Lee *et al.* 2001). The energy dissipation process by the inactivated RCs may be related to the charge recombination of $P680^+$ and the cyclic electron flow around the PS2 through cytochrome b_{559} (Whitmarsh *et al.* 1994).

Moreover, there has been a report that photoinhibition in soybean leaves is mostly due to the reversible inactivation of some PS2 RCs rather than a net loss of the D1 protein (Hong and Xu 1999a). A further investigation has demonstrated that the reversible inactivation of PS2 is linked to dissociation of LHC2 from PS2 complex (Hong and Xu 1999b). Based on a reversible dissociation of LHC2 from PS2 at temperatures above 35 °C, Sundby and Andersson (1985) assumed that the dissociation could be a short-term regulatory strategy for avoiding over-excitation and destruction of PS2 at high irradiance. However, there has been no direct experimental evidence for the assumption. In this study we obtained some results supporting the assumption.

Materials and methods

Plants: Soybean (*Glycine max* L. cv. Baimangjie) plants were grown in pots in a phytotron at a photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1} with 12/12 h light/dark cycle. Soybean cake was used as basal fertiliser. Fully expanded and healthy leaves (with F_v/F_m above 0.82) were used in experiments. Following dark-adaptation for 3 h, soybean leaves were cut from plants under water and the petioles of the leaves were put in water or 1 mM FSBA (*Sigma*) solution, which is an inhibitor of thylakoid protein kinase (Farchaus *et al.* 1985). These leaves were irradiated at 700 μmol(photon) m^{-2} s^{-1} (the saturating irradiance, SI, for photosynthesis of soybean grown in the phytotron) for 3 h. A halogen lamp (1 000 W) was used as light source. A flowing layer of water was between the lamp and the leaves to remove heat.

After the irradiation, the analysis of chlorophyll (Chl) fluorescence and measurement of PS2 electron transport activity of thylakoids from these leaves were made. For the D1 protein analysis and sucrose density gradient centrifugation these leaves were frozen and stored in liquid nitrogen after the irradiation.

Saturating irradiation of thylakoids: Soybean thylakoids were isolated from dark-adapted leaves according to Hong and Xu (1999a). The thylakoids were re-suspended to a Chl concentration of 0.4 kg m^{-3} in a buffer comprising 25 mM Tricine-NaOH (pH 7.8), 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 0.4 mM ATP. Some thylakoids were treated by FSBA (1 mM) at SI [700 μmol(photon) m^{-2} s^{-1}] and 25 °C for 12 min. Others without FSBA at SI and 25 °C were used as control. For the electron transport activity measurement, low-temperature Chl fluorescence analysis and the D1 protein detection, the thylakoids were harvested at the appropriate time of the irradiation.

Chl fluorescence: The initial fluorescence (F_0) and photochemical efficiency of PS2 (F_v/F_m) of dark-adapted soybean leaves at room temperature were measured with a portable Chl fluorometer *PAM-2000* (*H. Walz*, Effeltrich, Germany) as described by Hong and Xu (1999b). Low-temperature Chl fluorescence was mea-sured at 77 K with a 44 W-fluorescence spectrofluorimeter built in our laboratory, as described by Hong and Xu (1999b). During measurements, the excitation beam with the wavelength of 440 nm, provided by a 75 W tungsten lamp and a 440 nm filter, was focused on the leaf sample held at 77 K in a Dewar flask. The fluorescence signals were collected with a diode array detector, and the fluorescence spectrum between 650 and 750 nm was recorded by a dual-channel potentiometric recorder. Measurement of the fluorescence emission spectrum with the filter showed that the emission was essentially constant within the measuring range. The leaf sample holder was designed so that the excitation beam was of smaller size than the leaf sample itself. The built spectrofluorimeter has also been optimised for determinations of consistent yield at 77 K. Sample leaves with similar surface texture and at identical development stage and leaf position were used to minimise variations of the fluorescence yields caused by individual differences between leaves. It is thus possible to obtain reproducible fluorescence yields and determine absolute changes in 77 K fluorescence emission at 685 nm (F_{685}) and 735 nm (F_{735}).

Measurement of the PS2 electron transport activity in thylakoids: PS2 electron transport activity measurement of soybean thylakoids was performed according to Zhang *et al.* (1988) with some modification. Thylakoids were isolated from soybean leaves according to Hong and Xu (1999a). Chl content of the soybean thylakoid preparation was adjusted to 0.2 kg m^{-3} with a medium containing 25 mM Tricine-NaOH (pH 7.8), 0.4 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. The activity was measured at SI [1 200 μmol(photon) m^{-2} s^{-1}] or weak irradiance [100 μmol(photon) m^{-2} s^{-1}] by a Clark-type electrode, assembled by Shanghai Institute of Plant Physiology, using 0.5 mM 1,4-BQ as electron acceptor and 20 mM NH_4Cl as uncoupler. Considering the difference among the repeated experiments, all electron transport activities were given as percentages of dark control.

Sucrose density gradient centrifugation was made according to Hong and Xu (1999b) with some modification. Chl content of thylakoid membrane preparation was 2 kg m^{-3}. The membrane preparation was mixed with an equal volume of 2 % *n*-dodecyl-β-D-maltoside (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 sucrose linear 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 SW40* rotor at 650 rps and 4 °C for 16 h. Individual green layers were harvested with a syringe and stored at –20 °C.

SDS-PAGE and immunoblotting: Thylakoids were isolated from soybean leaves according to Hong and Xu (1999a) and all solutions contained 1 mM PMSF (phenylmethane-sulfonyl fluoride; *Merck*, Germany) to inhibit protease activity. Thylakoid membrane proteins were resolved by SDS-PAGE according to Laemmli (1970) using 15 % separating gel with 6 M urea. For immunoblotting, the polypeptides were transferred from gel of the SDS-PAGE to nitrocellulose membrane (*Amersham Pharmacia*, Buckinghamshire, England). Thylakoid phosphoproteins were immunodetected with a commercial polyclonal phosphothreonine antibody (*Zymed Laboratories,* USA) using the ECL assay kit (*Amersham Pharmacia*, England). The D1 proteins were detected with an anti-D1 antibody (a kind gift from Prof. Jian-Ren Shen). For quantification of the D1 proteins, the immunoblots were scanned with a laser densitometer (*Gel-Doc*, *Bio-Rad*, USA).

Results

Changes in low temperature fluorescence parameter F_{685}/F_{735} after FSBA treatment: Fig. 1*A* shows that the ratio of fluorescence yields at low temperature (77 K), F_{685}/F_{735}, declined significantly in leaves after SI compared to that of dark-adapted leaves. The decrease in F_{685}/F_{735} was mainly due to a significant decrease in F_{685} (the fluorescence yield from the antenna of PS2), because F_{735} (the fluorescence yield from the antenna of PS1) did not significantly change. FSBA treatment at SI could retard the decline in F_{685}. Similar results were obtained in *in vitro* experiments with isolated thylakoids (Fig. 1*B*).

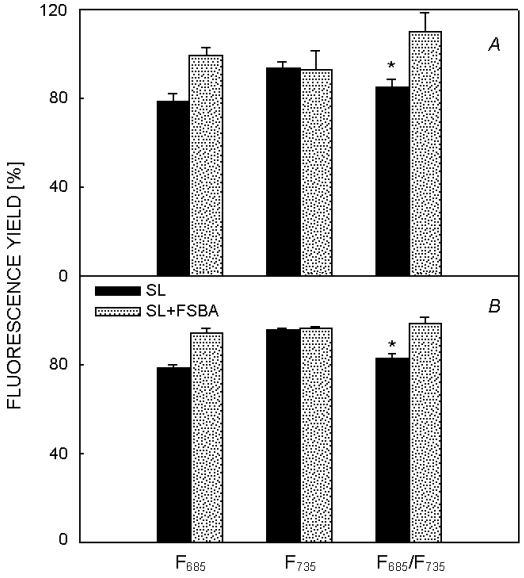

Fig. 1. Changes in low-temperature (77 K) chlorophyll fluorescence parameters F_{685}, F_{735}, and F_{685}/F_{735}, after saturating irradiation (700 μmol m^{-2} s^{-1}) in the absence and presence of FSBA in soybean leaves (*A*) and thylakoids (*B*). Means of 4–5 repeats with standard error expressed as bars. *difference between FSBA treatment and control is significant (p<0.05).

These results may imply that SI leads to dissociation of some LHC2s from PS2 complex, while FSBA treatment inhibits the dissociation.

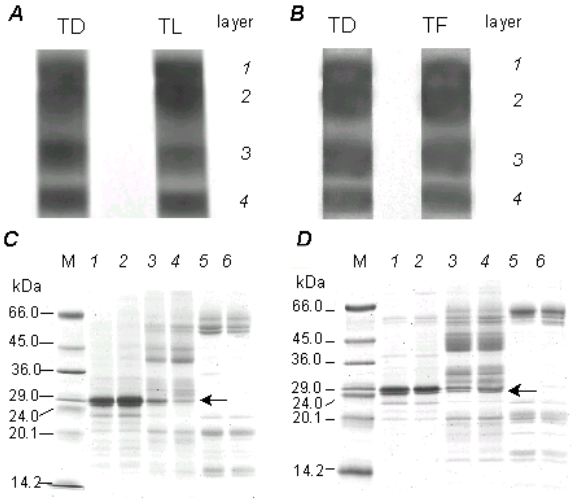

Fig. 2. Sucrose density gradient centrifugation of soybean thylakoid samples (*A, B*). TD – thylakoids from dark-adapted leaves; TL – thylakoids from saturating irradiance (SI) leaves (*A*); TF – thylakoids from FSBA-treated leaves at SI (*B*). SDS-PAGE analysis of the thylakoid composition in different layers from *A* and *B* (*C, D*). M – standard protein marker; lanes *1, 3, 5* – from layers *1* and *2, 3*, and *4* of TD, lanes *2, 4, 6* – from layer *1* and *2*, *3*, and *4* of TL (*C*) or TF (*D*), respectively. *The arrows* show LHC2.

Effect of FSBA treatment on LHC2 dissociation from PS2 complex: In general, four green layers are observed after sucrose density gradient centrifugation of thylakoid membrane samples. Layer *4* contained mainly the PS1 complexes, and layer *3* contained the PS2-LHC2 supercomplex. Layers *2* and *1* usually consist of major and minor LHC2 pigment-protein complexes (Barbato *et al.* 1992).

Fig. 2 shows our results of the sucrose density gradient centrifugation of thylakoids isolated from dark-adapted (TD), SI-irradiated (TL), and the FSBA-treated (TF) soybean leaves. For thylakoid sample from the SI-leaves, the green colour in layer *4* had no obvious change, but the green colour in layer *3* became lighter, while the layers *1* and *2* became darker, compared with those for thylakoid sample from the dark-adapted leaves (Fig. 2*A*). Since the layers *1* and *2* were not clearly resolved from each other in our experiment, they were analysed as a whole in the SDS-PAGE. Results showed that the relative amount of LHC2 was decreased in the layer *3* and increased in the layers *1* and *2* (Fig. 2*C*) for thylakoid sample from the SI-leaves, compared with the TD sample. Hence some LHC2s dissociated from the PS2 complex.

However, the green colour in layers of *3*, *2*, and *1* and the LHC2 amounts of layers *3*, *2*, and *1* had no significant change for the thylakoid samples from FSBA-treated leaves, as shown by the results of the sucrose density gradient centrifugation (Fig. 2*B*) and SDS-PAGE (Fig. 2*D*). These results indicate that FSBA treatment inhibits the dissociation of some LHC2s from PS2 complexes.

Effects of FSBA treatment on Chl fluorescence: In the SI-leaves, an elevation of about 33 % in F_0 and a decline of 16 % in F_v/F_m were observed, compared with those of dark-adapted leaves (Table 1). After 3 h in the dark, 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. In the presence of FSBA, however, SI caused much larger increases in F_0 (about 73 %) and decreases in F_v/F_m (about 27 %). Moreover, after 3 h in the dark, the two fluorescence parameters could not recover to the levels of dark-adapted leaves (Table 1), indicating that FSBA treatment at SI leads to irreversible damages in the photosynthetic apparatus.

Changes in PS2 electron transport activity after FSBA treatment: As shown in Table 2, SI of leaves alone had no significant effect on the PS2 electron transport activity ($H_2O \rightarrow$ 1,4-BQ) of isolated thylakoids measured at SI, although it caused a significant decrease in the activity (to 86 % of the control) when measured at weak irradiance. FSBA treatment at SI, however, led to a significant decline (to about 45 % of the control) in the activity measured at SI. When isolated thylakoids instead of leaves were treated by FSBA at SI [700 μmol(photon) m^{-2} s^{-1}], the activity also showed a severe decline after SI for 12 min (Fig. 3*B*). Nevertheless, FSBA treatment in the dark of isolated thylakoids had no effect on the PS2 electron transport activity (Fig. 3*A*), indicating that FSBA itself does not inhibit the PS2 electron transport.

Table 1. Changes in chlorophyll fluorescence parameters in soybean leaves after 1 mM FSBA treatment at saturating irradiance (SI; 700 μmol m^{-2} s^{-1}). Means of 3-5 leaves ± standard error; the values in parentheses are the percentage of dark-adapted leaves (before SI). * and **, significant differences between FSBA-treated and dark control leaves for $p<0.05$ and $p<0.01$, respectively.

Treatment		SI	SI + FSBA
Before SI	F_0	0.263 ± 0.003 (100)	0.213 ± 0.002 (100)
	F_v/F_m	0.842 ± 0.001 (100)	0.839 ± 0.008 (100)
After 3 h SI	F_0	0.350 ± 0.014 (133.2)*	0.367 ± 0.011 (173.6)**
	F_v/F_m	0.705 ± 0.003 (83.7)*	0.605 ± 0.003 (73.0)**
After 3 h dark recovery	F_0	0.266 ± 0.007 (101.0)	0.322 ± 0.008 (152.0)*
	F_v/F_m	0.827 ± 0.006 (98.2)	0.683 ± 0.008 (82.4)*

Table 2. Effects of FSBA treatment [% of dark control] at saturating irradiance (SI) for 3 h on the photosynthetic electron transport activity of PS2 ($H_2O \rightarrow$ 1,4-BQ) in soybean thylakoids. The PS2 electron transport activities of dark control thylakoids measured at SI and at weak irradiance were 32-57 and 18-24 mmol(O_2) kg^{-1}(Chl) s^{-1}, respectively. * and **, significant differences between FSBA-treated and dark control leaves for $p<0.05$ and $p<0.01$, respectively.

Treatment	Measuring irradiance	Repeats 1	2	3	4	5	Mean
SI	SI	107.3	98.9	103.4	92.5	101.2	100.7 ± 2.5
	Weak irradiance	88.7	86.2	84.4	—	—	86.4 ± 1.2*
SI+FSBA	SI	43.1	42.8	49.6	—	—	45.2 ± 2.2**

Changes in the D1 protein amount after FSBA treatment: The Western blot reaction of the D1 protein had a linear response to an increase in the amount of thylakoid samples loaded on the gel (Fig. 4*A,B*). Therefore, it can be used for relative quantification of the D1 proteins. After SI, the amount of the D1 proteins in soybean leaves remained similar to that of dark-adapted leaves (Fig. 4*C,D*), indicating that no net loss of the D1 proteins occurs in soybean leaves. However, the FSBA treatment at SI caused a loss of about 39 % in the D1 protein amount

(lane *3*), as compared with that of SI-leaves without FSBA (lane *2*) and dark control (lane *1* in Fig. 4*C,D*). When the isolated thylakoids were treated by FSBA at SI *in vitro*, a decrease of about 48 and 16 % in the D1 protein amount occurred in the presence and absence of FSBA, respectively (Fig. 5). These results are consistent with those from leaves.

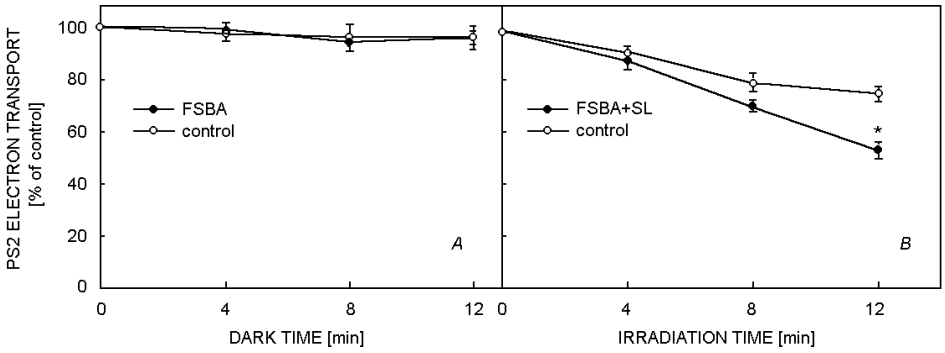

Fig. 3. Effect of FSBA treatment on photosystem 2 (PS2) electron transport activity of soybean thylakoids measured at saturating irradiance (SI) and 25 °C. (*A*) FSBA treatment in the dark; (*B*) FSBA treatment at SI. Means of 3-4 repeats with standard error expressed as bar. *significant differences between FSBA-treated and dark control thylakoids (p<0.05).

Changes in phosphorylation level of PS2 core and LHC2 proteins after FSBA treatment: According to Rintamäki *et al.* (1997) the main phosphoprotein bands of pumpkin thylakoid membranes are PS2 core proteins (CP43, D2 and D1) and LHC2 proteins as recognised by the Thr (P) antibody. However, the phosphorylations of PS2 core proteins and LHC2 proteins *in vivo* were diffeently regulated in response to high irradiance (Rintamäki *et al.* 1997). Similar results were also obtained in our experiment. In the soybean thylakoid membranes, the phosphoproteins identified by the antibody in SDS-PAGE were P-CP43, P-D2, P-D1, and P-LHC2 (Fig. 6). In the absence of FSBA, SI for 3 h led to an increase in the phosphorylation level of PS2 core proteins (D1, D2, and CP43) and a reduction in phosphorylation level of LHC2 proteins. In the presence of FSBA, it resulted in marked declines in the phosphorylation level of the two kinds of proteins, especially in that of the PS2 core proteins. These results indicate that FSBA inhibits the phosphorylation of thylakoid membrane proteins, especially of PS2 core proteins (Fig. 6).

Discussion

Inhibition of LHC2 dissociation from PS2 complex by FSBA treatment: We found that LHC2 dissociation from PS2 complex occurs under SI and that FSBA treatment can inhibit the dissociation in soybean leaves. The following facts support the viewpoint:

(*1*) A significant decrease in F_{685} was observed after SI without FSBA, however, the decrease could be retarded by FSBA (Fig. 1). At 77 K the Chl fluorescence peaking 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 photon energy absorbed by LHC2 can be transferred to the core antenna providing that the LHC2 is associated with the 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.

Of course, because there are disagreements in recognising F_{685}, the LHC2 dissociation may not be the sole explanation of the change in F_{685}. Other events, for example, the degradation of LHC2, can also lead to a decrease in F_{685}. However, the rapid recovery of F_{685} and F_{685}/F_{735} in the dark rules out the possibility of LHC2 degradation (Hong and Xu 1999b, Cai and Xu 2002). Furthermore, NaF, a phosphatase inhibitor, can inhibit the dark recovery of fluorescence parameters F_v/F_m, F_0, and F_{685} (Hong and Xu 1999b). This fact can not be explained by *de novo* LHC2 synthesis and indicates that the recombination of dissociated LHC2 plays a key role in the dark recovery of PS2 function.

(*2*) In the 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 thylakoids from SI-leaves, as compared with those for the thylakoids from dark-adapted leaves (Fig. 2*A*). As shown by SDS-PAGE, moreover, the relative amount of LHC2 decreased

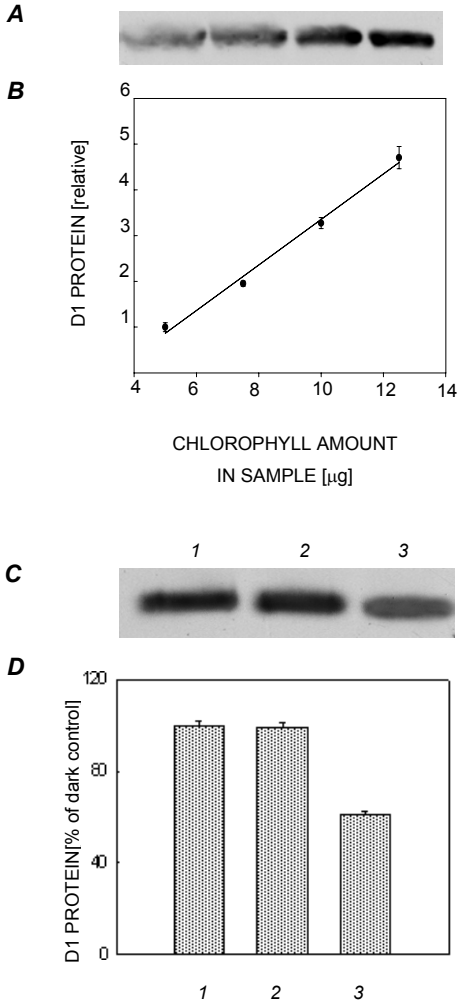

Fig. 4. Effect of FSBA treatment at saturating irradiance (SI) for 3 h on the quantity of the D1 proteins in soybean leaves. (*A*) The linearity of immunoresponse of D1 antibody to D1 protein. (*B*) Quantitative demonstration of the linear response by a *Gel-Doc* laser densitometer. (*C*) Immunoblot of the D1 proteins, the samples containing 7.5 μg chlorophyll were loaded on the gel. (*D*) Qualitative demonstration of the D1 proteins by a *Gel-Doc* laser densitometer. Lane *1* – dark control leaves; lane *2* – SI-leaves; lane *3* – SI+FSBA-treated leaves. Means of 3 repeats with standard errors expressed as bars.

in the layer *3* and increased in the layers *1* and *2* (Fig. 2*C*), indicating that some LHC2s dissociated from the PS2 complex. However, such changes in the sucrose density gradient centrifugation and SDS-PAGE did not occur for thylakoids from the FSBA-treated leaves at SI (Fig. 2*B,D*), indicating that the LHC2 dissociation is inhibited by FSBA.

(*3*) The electron transport activity of PS2 did not significantly change in the thylakoids from SI-leaves when measured at SI, however, it substantially declined when measured at weak irradiance (Table 2). The discrepancy of change in the electron transport activity measured at different irradiances results only from the dissociation of LHC2. Dissociation of some LHC2s leads to a decrease in the photon absorption cross-section of PS2 and thereby a decrease in the excitation energy transferred to the PS2 RCs. Thus, when the measuring irradiance is limited, the smaller antenna and thereby less photon energy transferred to PS2 RCs lead to a decrease in the charge separation rate of the centres, and thereby a substantial decline in the electron transport activity. When the measuring irradiance is saturated, however, the dissociation of some LHC2s cannot induce the decline in the PS2 electron transport activity because the excessive photons can compensate the reduction in light-harvesting cross-section.

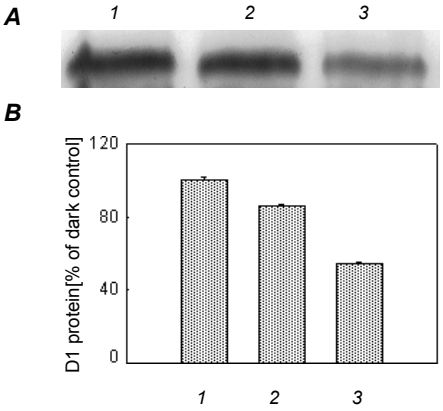

Fig. 5. Effect of FSBA treatment at saturating irradiance (SI) for 12 min on the quantity of D1 protein in soybean thylakoids. (*A*) Immunoblot of the D1 proteins, the samples containing 7.5 μg chlorophyll were loaded on the gel. (*B*) Qualitative demonstration of the D1 proteins by a *Gel-Doc* laser densitometer. Lane *1* – dark control thylakoids; lane *2* – SI-thylakoids; lane *3* – SI+FSBA-treated thylakoids. Means of 3 repeats with standard errors expressed as bars.

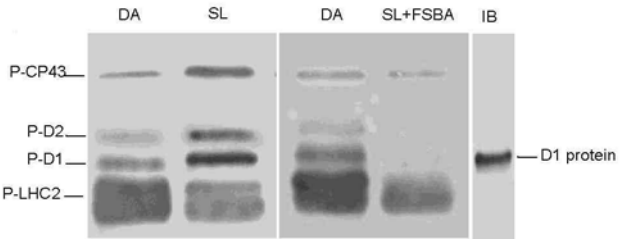

Fig. 6. Effect of saturating irradiance (SI) on the phosphorylation level of soybean thylakoid membrane proteins in the absence and presence of FSBA. Thylakoid membranes were isolated from dark-adapted (DA) and SI-leaves. An immunoblot (IB) of thylakoid membrane protein with D1 antibody shows the position of D1 protein migration.

Protective role of LHC2 dissociation against photodamage to PS2 RCs: Although there has been no direct experimental evidence for the assumption about the protective role of LHC2 dissociation (Sundby and Andersson 1985), the following facts support the assumption:

(*1*) The extents of both increase in F_0 and decrease in F_v/F_m were much larger after SI in the presence than in the absence of FSBA. And after dark recovery for 3 h, the two parameters could recover to the levels of dark control in the SI-leaves without FSBA, but not in the SI-leaves with FSBA (Table 1). These irreversible changes in F_0 and F_v/F_m of the FSBA-treated leaves indicate the occurrence of irreversible photodamage of some PS2 RCs.

(*2*) The irradiance-saturated PS2 electron transport activity had no significant change for the thylakoids from the SI-leaves without FSBA, while it decreased significantly for those from FSBA-treated leaves at SI (Table 2). The significant decrease in the activity is only due to a function loss of some PS2 RCs because FSBA itself has no effect on the PS2 electron transport activity (Fig. 3*A*, also see Farchaus *et al.* 1985).

(*3*) The amount of D1 proteins in SI-irradiated soybean leaves in the absence of FSBA did not change (Fig. 4, lane *2*), but it significantly decreased in SI-leaves in the presence of FSBA (Fig. 4, lane *3*). Furthermore, FSBA treatment *in vitro* of isolated thylakoids also caused a larger decline in the D1 protein amount during SI (Fig. 5). The reason that a net loss of the D1 proteins was observed *in vitro* but not *in vivo* may be the inability of isolated thylakoids to synthesise replacement D1 proteins, thereby an unbalance occurs between the degradation and the synthesis of the D1 protein (Ohad *et al.* 1985, 1990). It is often thought that photodamage of PS2 complex results from a net loss of the D1 proteins (Ohad *et al.* 1990). Therefore, the significant decrease in the D1 protein amount is an indication of damage of some PS2 RCs.

All these facts suggest that at SI some LHC2s can dissociate from PS2 complex and the LHC2 dissociation is an ideal strategy protecting PS2 RCs against over-excitation and photodamage. At high irradiance, the dissociation of LHC2 may decrease the size of antenna and the amount of excitation energy transferred to the RC. After irradiance stress is removed, dissociated LHC2 may rejoin PS2 complex, increasing light-harvesting cross-section. The excessive excitation energy can be safely dissipated as heat within the light-harvesting antenna of PS2 (Elrad *et al.* 2002, Govindjee 2002) and in the thermal dissipation the PsbS protein is important (Li *et al.* 2000, 2002). So we can deduce that the dissociated LHC2s may dissipate the excessive excitation energy and thereby protect the PS2. However, the mechanism for the thermal dissipation by the dissociated LHC2 and the relationship between LHC2 dissociation and PsbS protein are yet worth further investigation.

Allowing for the unspecific effect of FSBA on the kinases, there is a possibility that the photodamage of some PS2 RCs is caused by the inhibition of ATP synthesis and/or the Calvin cycle rather than the inhibition of LHC2 dissociation. When the leaves are used in experiment, all cells, chloroplasts, and enzyme systems of photosynthesis are intact. The processes of both ATP synthesis and carbon assimilation can operate normally. So FSBA treatment *in vivo* can inhibit the ATP synthesis and/or some kinases of the Calvin cycle, leading to photodamage to PS2 RCs at SI. However, this possibility can be excluded by a thylakoid experiment *in vitro*. In our thylakoid experiment system with uncoupler (20 mM NH_4Cl), no enzyme system of the Calvin cycle existed, and neither ATP synthesis nor carbon assimilation could occur. Therefore, the photodamage of some PS2 RCs in the presence of FSBA at SI, as shown by Figs. 3 and 5, is caused only by the inhibition of LHC2 dissociation rather than the inhibition of ATP synthesis and carbon assimilation.

Key role of PS2 core protein phosphorylation in LHC2 dissociation: An earlier investigation indicated that phosphorylation of thylakoid proteins plays important role for the dissociation of LHC2 from PS2 complex (Hong and Xu 1999a,b). The present results suggest that phosphorylation of PS2 core proteins rather than LHC2 proteins play a critical role in the dissociation of LHC2. First, when the dissociation of LHC2 occurred, as shown by the analysis of the low-temperature (77 K) Chl fluorescence (Fig. 1) and sucrose density gradient centrifugation (Fig. 2), phosphorylation level of LHC2 proteins was reduced, while that of PS2 core proteins, including D1, D2, and CP43, increased (Fig. 6). The phosphorylation of LHC2 proteins is important in the LHC2 detachment from PS2 during state transition (Anderson and Andersson 1988, Allen 1992a,b). Nevertheless, the decline in phosphorylation level of LHC2 proteins after SI implies that the phosphorylation of LHC2 proteins is not a key driving force for the LHC2 dissociation at excessive irradiance reported here. And the dissociation of LHC2 in soybean leaves is different from that during state transition (Hong and Xu 1999b). Second, when the phosphorylation of PS2 core proteins was inhibited by FSBA (Fig. 6), the dissociation of LHC2 from PS2 complex was also inhibited (Fig. 1). The phosphorylation of PS2 core proteins can induce changes in the structure of PS2 core (He *et al.* 1991). Perhaps these changes lead to a loose bounding of LHC2 with PS2 RC complex, thereby to the dissociation of LHC2.

Several protective mechanisms against photodamage to the photosynthetic apparatus may operate simultaneously in higher plants exposed to excessive photons. Our results indicate that the dissociation of LHC2 from PS2 complex is an effective protective strategy against over-excitation and photodamage of the PS2 RCs in soybean leaves. The questions whether the strategy acts in other plant species and how the excitation energy absorbed by the dissociated LHC2 is dissipated as heat are worth studying.

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