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Inhibition of photosynthesis by shift in the balance of excitation energy distribution between photosystems in dithiothreitol treated soybean leaves

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Abstract

Chlorophyll fluorescence kinetics was used to investigate the effect of 1,4-dithiothreitol (DTT) on the distribution of excitation energy between photosystem 1 (PS1) and photosystem 2 (PS2) in soybean leaves under high irradiance (HI). The maximum PS2 quantum yield (F_v/F_m) was hardly affected by the presence of DTT, however, photon-saturated photosynthesis was depressed distinctly. Photochemical efficiency of open PS2 reaction centres during irradiation (F_v'/F_m') was enhanced by about 30–40 % by DTT treatment, whereas photochemical quenching (q_P) was depressed by about 40 % under HI. DTT treatment caused a 30 % decrease in allocation of excitation energy to PS1 under HI and a 20 % increase to PS2. An obvious shift in the balance of excitation energy distribution between photosystems was observed in DTT-treated leaves. Though high excitation pressure ($1 - q_P$) resulted from DTT treatment, non-photochemical quenching (q_N) was lower. DTT completely inhibited the formation of zeaxanthin and also distinctly depressed the state transition (q_T). The shift in the balance of excitation distribution between the two photosystems induced by DTT was mainly due to the enhancement of excitation energy capture by PS2 antenna and the inhibition of state transition. It might be the shift in the balance between the two photosystems that mainly induced the depression of photosynthesis. Thus, to keep high utilization efficiency of absorbed photon energy, it is necessary to maintain the balance of excitation distribution between PS2 and PS1.

Additional key words: chlorophyll fluorescence; excitation energy distribution; net photosynthetic rate; photosystems; quenching; state transition; xanthophyll cycle.

Introduction

Leaves grown in HI often absorb more energy than can be utilized in photosynthetic electron transport. The excessive excitation energy would lead to inactivation or photodamage of photosystem 2 (PS2), if it was not safely dissipated by PS2 antennae (Demmig-Adams 1990, Li *et al.* 2000). The efficiency of light-harvesting by PS2 in higher plants is subject to regulation (Demmig-Adams and Adams 1992, Horton and Ruban 1992). These regulation mechanisms located in antennae can efficiently balance the photon capture and utilization (Osmond 1994, Demmig-Adams *et al.* 1995).

Oxygenic photosynthesis relies on the function of two distinct types of membrane-embedded photochemical reaction centres, termed photosystem 1 (PS1) and photosystem 2 (PS2), which operate in series. PS1 is mainly located in the stroma lamellae and PS2 is mainly located in the appressed grana stacks (Anderson and Melis 1983, Anderson 1999). Each photosystem has its own antenna, which implies a spectral unbalance of the exciting irradiance that may result in an unbalanced excitation of the two photosystems (Fork and Satoh 1986, Malkin *et al.* 1986). State transition is one of the important mechanisms regulating excitation transfer between the two photosystems (Wollman 2001), which was discovered about 30 years ago in unicellular photosynthetic organisms. This short-term regulation (state transition) is based on the reversible phosphorylation of light-harvesting complex 2 by a thylakoid-bound kinase (Allen *et al.* 1981, Rintamäki *et al.* 1997, Carlberg *et al.* 1999, Depege *et al.* 2003). Under irradiance, the kinase phosphorylates subunits of the light-harvesting complexes 2 (LHC2) and the phosphorylated LHC2 dissociates from PS2 to PS1, thus the efficiency of energy harvesting by PS2 is lowered (Allen *et al.* 1981, Depege *et al.* 2003).

Another important mechanism that can regulate the efficiency of energy harvesting in PS2 complex is

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xanthophyll cycle (Horton *et al.* 1996, Gilmore 1997). The de-epoxidised xanthophyll cycle components zeaxanthin (Z) and antheraxanthin (A) facilitate the thermal dissipation of excessive excitation energy in PS2 antennae (Gilmore and Yamamoto 1993, Horton *et al.* 1996, Gilmore 1997). This xanthophyll cycle-dependent dissipation process lowers the efficiency of open PS2 units through a decrease in excitation energy transfer from the LHC2 to the reaction centres (Gilmore 1997). This down-regulation in PS2 efficiency can balance the delivery of excitation energy to PS2 with the rate of consumption of electron transport products in metabolic reactions thereby avoiding the accumulation of excess excitation energy in pigment bed. Recent experiments demonstrated that CP22, a minor protein of the PS2 reaction centre, may be the site of xanthophyll cycle-dependent excitation energy dissipation; or CP22 may be necessary to maintain LHC2 proteins in a proper super-molecular organization that allows xanthophyll cycle-dependent NPQ to occur (Li *et al.* 2000).

Generally, the formation of Z can be completely prevented in leaves, by pre-treatment with the sulfhydryl reagent DTT (1,4-dithiothreitol) which inhibits the violaxanthin de-epoxidase (Yamamoto and Kamite 1972, Horton *et al.* 1994). Hence DTT has usually been presented as demonstrating the participation of Z in non-photochemical quenching. If energy dissipation depending on xanthophyll cycle was inhibited, excessive energy might accumulate in PS2. To keep balance in excitation energy distribution between PS1 and PS2, will state transitions in DTT feeding leaves play a more important role in excessive energy distribution? Will the captured excitation energy be kept in balance between PS1 and PS2 in the presence of DTT under strong irradiance? If not, what will happen to photosynthesis? In this study, we used soybean to investigate these questions.

Materials and methods

Plants and growth conditions: Potted soybean (*Glycine max* L. cv. Ludou 11) plants were grown in field for 1 month in the summer of 2003 under natural irradiation with a daily maximum photosynthetically photon flux density (PPFD) of about 1 400 µmol m^{-2} s^{-1}. Young and fully expanded leaves were used for the experiments.

Net photosynthetic rate (P_N) was measured at room temperature (25 °C) and 85 % relative humidity with a portable system (*CIRAS-1*, *PP Systems*, UK). P_N–PPFD relationship was measured while PPFD was changed every 10 min in a sequence of 2 000, 1 600, 1 200, 800, 600, 400, 300, 200, 150, 100, and 0 µmol m^{-2} s^{-1}. PPFD was controlled by the automatic control device of the *CIRAS-1* photosynthetic system.

Uptake of inhibitor: Leaf petioles were cut under water and transferred to small tubes containing distilled water (control, CK) or 10 mM DTT solution (Park *et al*. 1995, Darkó *et al*. 2000). They were allowed to take up water or DTT for about 5 h under weak irradiance (30 µmol m^{-2} s^{-1}). The DTT content in the bulk leaf tissue was determined according to Bilger and Björkman (1994). The solution taken up was monitored periodically and the content of DTT in the bulk leaf tissue, C_L, was estimated from the expression $C_L = V_S C_S/FM_L$, where V_S is the volume taken up, C_S is the DTT concentration of the solution, and FM_L is the fresh mass of the leaf. Considering the relative water content of leaf is greater than 90 % in well watered condition, FM_L can be regarded as the bulk of leaf because the density of water is 1. The average concentration of DTT in leaf tissue was about 5–6 mM.

Chlorophyll (Chl) fluorescence was measured using a Pulse-Modulated Fluorimeter (*FMS2*, *Hansatech*, UK). The maximum PS2 quantum yield (F_v/F_m) was determined in dark-adapted (15 min) samples. After the initial Chl fluorescence yield (F_0) was determined under low modulated measuring radiation, a 0.7-s pulse of saturating "white light" (>3 000 µmol m^{-2} s^{-1}) was applied to obtain the maximum Chl fluorescence yield (F_m) and the F_v/F_m (F_v, the variable Chl fluorescence yield, is defined as $F_m - F_0$). The fluorescence transient was induced by continuous actinic "white light" (1 400 µmol m^{-2} s^{-1}) for 50 min, provided by the fluorimeter radiation source. The photochemical fluorescence-quenching (q_P) and non-photochemical quenching (q_N) coefficients were distinguished by applying saturating pulses during the last 5 min of the continuous irradiation. After the "actinic light" had been switched off, 2-s far-red radiation was applied for determination of the minimal fluorescence (F_0'). Photochemical efficiency of open PS2 reaction centres during irradiation (F_v'/F_m') and q_P were calculated as $(F_m' - F_0')/F_m$ and $(F_m' - F_s)/(F_m' - F_o')$, respectively (Genty *et al.* 1989). q_N was calculated as $1 - (F_m' - F_o')/(F_m - F_o)$ (van Kooten and Snell 1990). α and β, the photon distribution coefficients of PS1 and PS2, were determined according to Malkin *et al*. (1986) and Braun and Malkin (1990). State transitions (q_T) were measured by analyzing the relaxation of NPQ during dark period (Quick and Stitt 1989).

Pigment analysis: After dark adaptation for 12 h, the soybean leaves were exposed to irradiance of 1 400 µmol m^{-2} s^{-1} for 0 and 50 min, and then were quickly frozen in liquid nitrogen. Leaf samples were extracted with 100 % acetone. Pigments were separated using HPLC system (*Waters*, USA) at room temperature according to Thayer and Björkman (1990) with some modifications. A two-solvent gradient program was used, solvent mixtures were: *A*: 0.05 M acetonitrile–methanol–Tris–HCl (75 : 15 : 10, v : v : v), *B*: methanol–hexane (5 : 1).

Solvent *A* was run isocratically for 5 min with a rate of 33.33 $mm^3\ s^{-1}$. Solvent *B* was then run isocratically for 10 min. The columns were re-equilibrated between samples for 15 min with solvent *A*, at a flow rate of 16.66 $mm^3\ s^{-1}$. 10 mm^3 samples were injected and the absorbance at 440 nm was recorded. Concentration of the pigments was estimated by using the conversion factors for peak area to nmol as determined for this solvent mixture by Thayer and Björkman (1990).

Results

Effects of DTT on the maximum efficiency of PS2 photochemistry and photosynthesis: The initial Chl fluorescence yield (F_0), the maximum Chl fluorescence yield (F_m), and the maximum PS2 quantum yield (F_v/F_m) of the leaves were hardly effected by the DTT treatment (Table 1). After 50 min irradiance by 1 400 μmol $m^{-2}\ s^{-1}$, little difference in F_v/F_m was also observed between CK and DTT treated leaves during their dark recovery period (Fig. 1), indicating that DTT treatment did not lead to inactivation of PS2. However, DTT had an obvious influence on photosynthesis (Fig. 2). The PPFD-saturated P_N values in CK and DTT-treated soybean leaves were about 16 and 12 μmol $m^{-2}\ s^{-1}$, respectively; hence about 25 % reduction was induced by DTT treatment (Fig. 2).

Table 1. Effects of 5 mM dithiothreitol (DTT) on the initial chlorophyll fluorescence yield (F_0), the maximum chlorophyll fluorescence yield (F_m), the variable chlorophyll fluorescence yield (F_v), and the maximal efficiency of PS2 photochemistry (F_v/F_m) in dark-adapted soybean leaves. CK = control.

Treatment	F_0	F_m	F_v	F_v/F_m
CK	244±11	1663±46	1419±32	0.853±0.020
+DTT	264±12	1657±49	1393±35	0.841±0.030
+DTT/CK	108.0	99.6	98.0	98.6

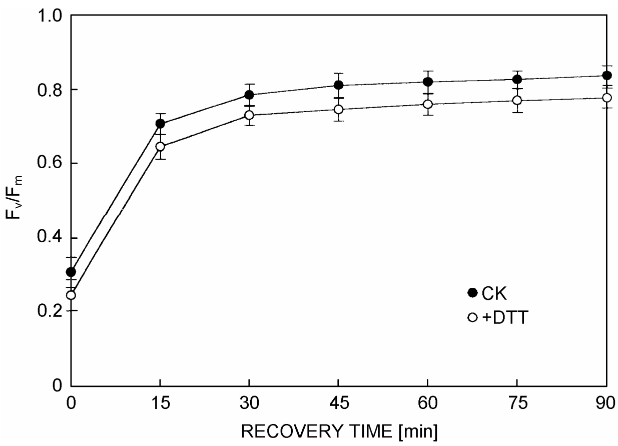

Fig. 1. Recovery courses of the maximal efficiency of PS2 photochemistry (F_v/F_m) under weak irradiance (30 μmol $m^{-2}\ s^{-1}$) in control (CK) and DTT-treated leaves after being treated for 50 min with strong irradiation of 1 400 μmol $m^{-2}\ s^{-1}$. Chlorophyll fluorescence was measured at room temperature (25 °C). *Bars* indicate SE (*n* = 3).

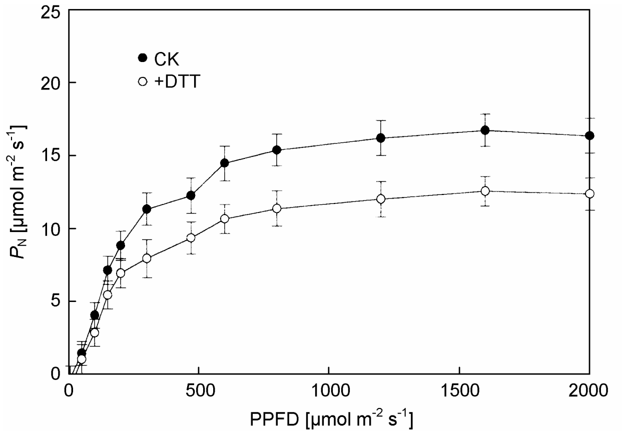

Fig. 2. Response curves of net photosynthetic rate (P_N) to irradiance (PPFD) in control (CK) and DTT-treated soybean leaves measured in 340 μmol(CO_2) mol^{-1} at room temperature (25 °C).

Effects of DTT on the photochemical efficiency of open PS2 centres in the presence of non-photochemical quenching (F_v'/F_m') and photochemical quenching (q_P): F_v'/F_m' reflects the antenna efficiency at open PS2 centres in the presence of non-photochemical quenching under irradiation (Genty *et al.* 1989, Gilmore 1997). When exposed to 1 400 μmol $m^{-2}\ s^{-1}$ irradiance, the values of F_v'/F_m' in CK leaves were low, however, in leaves fed with DTT this parameter stayed rather high (Fig. 3*A*). Compared with control leaves, DTT treatment enhanced F_v'/F_m' by about 30–40 %. After 50-min HI exposure, q_P both in CK and DTT-treated leaves were about 0.58 and 0.34, respectively (Fig. 3*B*), thus a 40 % decrease was observed in the DTT treated leaves.

Effects of DTT on excitation energy distribution between PS1 and PS2: Malkin *et al.* (1986) developed a method that can quantitatively estimate the excitation energy distribution between PS1 and PS2. Under irradiance of 1 400 μmol $m^{-2}\ s^{-1}$, excitation energy allotted to PS2 (β) in DTT treated leaves was 20 % higher than that in CK leaves; whereas, the excitation energy distributed to PS1 (α) in the presence of DTT was 30 % lower compared with that in control (Fig. 3*C,D*). Hence more excitation energy was distributed to PS2 in DTT treated leaves, indicating that DTT treatment might lead to a shift in the balance of excitation energy distribution between PS1 and PS2. By calculating the deviation of excitation energy from full balance between PS1 and PS2 ($\beta/\alpha - 1$), it was proved that under HI, DTT caused a serious shift in the balance (Fig. 4*A*).

Effects of DTT on excitation pressure of PS2 and q_N: PS2 excitation pressure ($1 - q_P$) reflects the redox state of PS2 and is a convenient and sensitive indicator of the balance between energy supply and its utilization (Van Kooten and Snell 1990, Schreiber *et al.* 1994). In DTT poisoned leaves, $1 - q_P$ was higher than that in CK leaves, indicating that DTT treatment led to higher excitation pressure of PS2 compared with that in control (Fig. 4*B*).

q_N reflects excessive energy dissipation, which can be monitored by Chl *a* fluorescence. In the presence of DTT, q_N was drastically inhibited compared with control (Fig. 4*C*). Since the major component of q_N in most plants depends on the xanthophyll cycle (Briantais *et al.* 1980, Björkman 1987, Li *et al.* 2000), components of the cycle were examined by HPLC. Obviously, the formation of Z was completely suppressed by DTT under 1 400 μmol m^{-2} s^{-1} irradiance for 50 min; however, the formation of A was not influenced (Table 2).

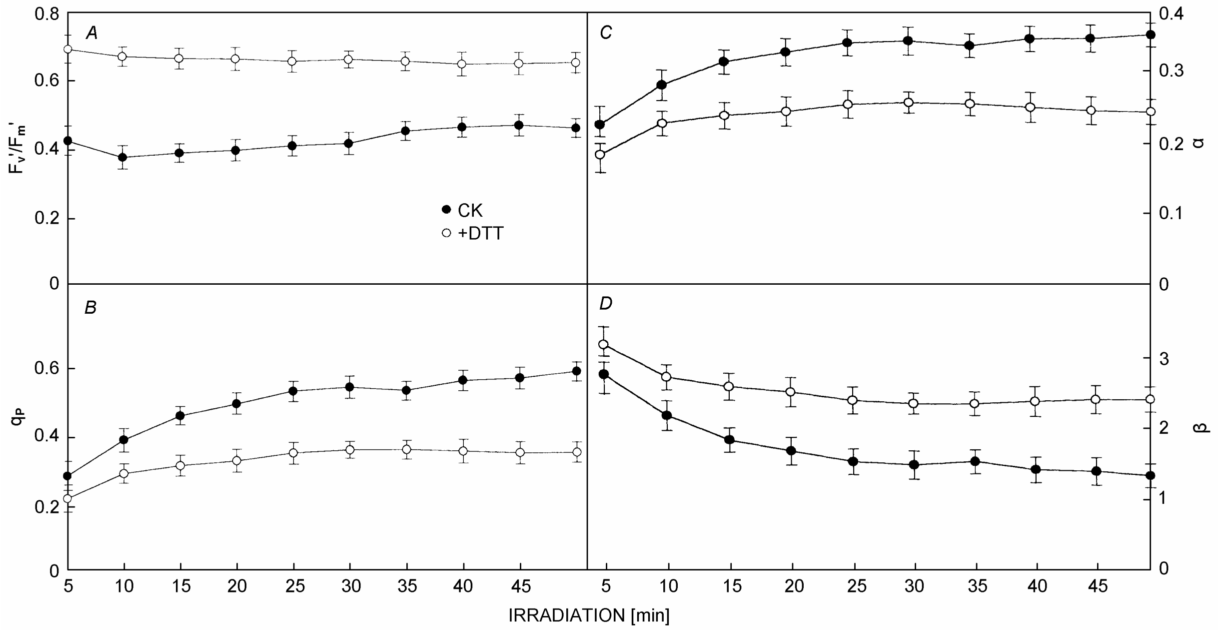

Fig. 3. Effect of DTT on the antenna efficiency at open reaction centres in the presence of non-photochemical quenching, F_v'/F_m' (*A*), on photochemical quenching, q_p (*B*), and on excitation energy distribution to PS1, α (*C*) and PS2, β (*D*) in soybean leaves exposed to PPFD of 1 400 μmol m^{-2} s^{-1}. Measured in 340 μmol(CO_2) mol^{-1} at room temperature (25 °C). *Bars* indicate SE (*n* = 3). CK = control.

Table 2. Effect of DTT on the contents of the three components of the xanthophyll cycle [mmol mol^{-1}(Chl)] in soybean leaves in darkness or under an irradiance of 1 400 μmol m^{-2} s^{-1} for 50 min. A = antheraxanthin, V = violaxanthin, Z = zeaxanthin, CK = control.

Treatment		Z	A	V	Z+A+V	[Z/(Z+A+V)] [%]
CK	Dark	0±1	4.3±2.0	85.6±8.0	89.9±10.0	0±1
	Light	28.4±4.0	17.6±3.0	49.5±6.0	95.5±9.0	30±3
+DTT	Dark	0±1	3.8±2.0	86.3±8.0	90.1±7.0	0±1
	Light	0±1	18.2±4.0	73.8±6.0	92.0±11.0	0±1

State transition is another important mechanism regulating excitation distribution (Wollman 2001). Measurement of the state transition under HI revealed a significant suppression by DTT (Fig. 4*D*).

Discussion

The changes of energy distribution coefficients of PS1 and PS2 (α and β) in the presence of DTT reflected the shift in the balance of excitation energy distribution between PS1 and PS2 (Figs. 3*C,D* and 4). The same conclusion can also be made by analysis of PS2 excitation pressure (Fig. 4*B*). Xanthophyll cycle is a very important mechanism to dissipate excitation energy of PS2, which can effectively alleviate the excitation pressure of

PS2 (Horton *et al.* 1996, Gilmore 1997). Values in Table 2 demonstrate that the formation of Z under HI was completely suppressed by DTT, though the formation of A was not inhibited (Table 2). A is also important in the dissipation of excitation energy (Goss *et al.* 1998), which may partly explain why there was still a fraction of q_N in DTT treated leaves (Fig. 4*C*). The inhibition of xanthophyll cycle by DTT led to less dissipation of excitation energy in the antenna and higher efficiency of excitation capture by open PS2 centres (Fig. 3*A*), thereby resulting in an increased flow of excitation energy to the PS2 centres and higher reduction of Q_A (Fig. 4*B*).

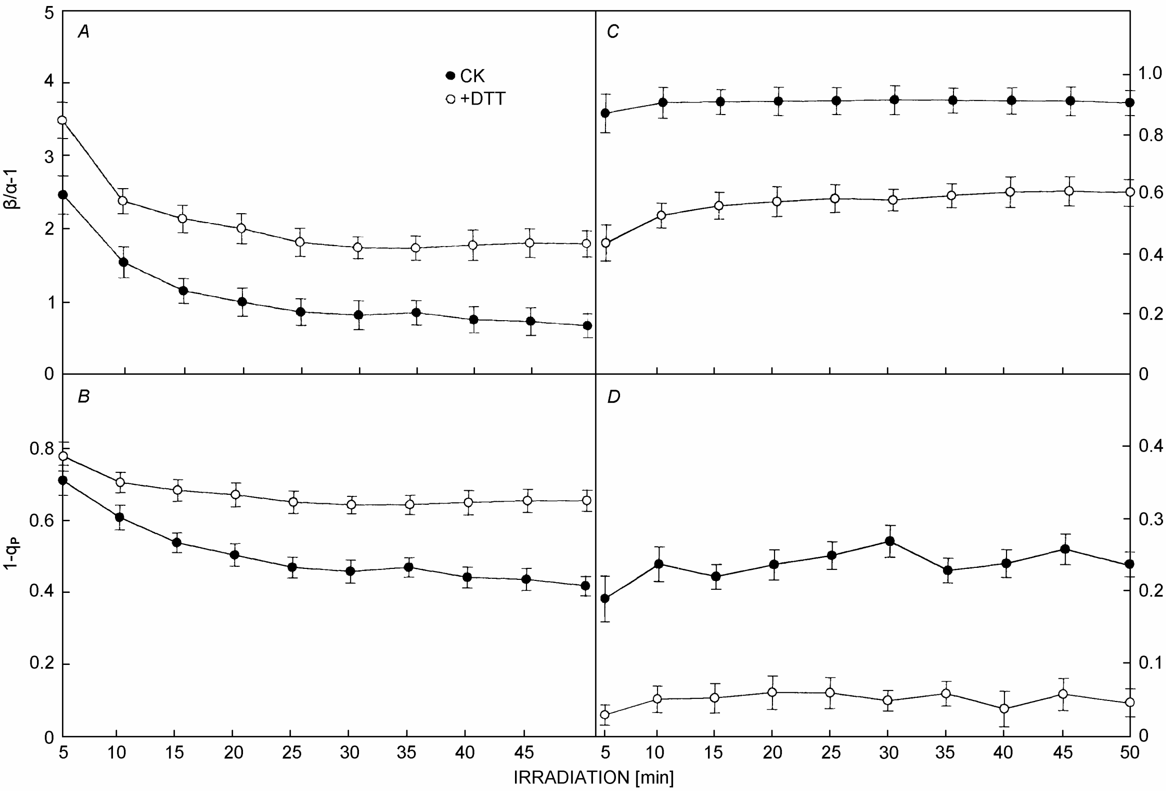

Fig. 4. Effect of DTT on the deviation of excitation energy from full balance between photosystems 1 and 2, $\beta/\alpha - 1$ (*A*), reduction state of Q_A, $1 - q_p$ (*B*), non-photochemical quenching, q_N (*C*), and state transition, q_T (*D*) in soybean leaves exposed to a PPFD of 1 400 µmol m^{-2} s^{-1}. Measured in 340 µmol(CO_2) mol^{-1} at room temperature (25 °C). *Bars* indicate SE (*n* = 3). CK = control.

State transition is another important mechanism, which regulates excitation transfer between the two photosystems and alleviates PS2 excitation pressure (Demmig and Winter 1988, Ruban and Horton 1995, Wollman 2001). However, the state transition in soybean leaves treated with DTT was distinctly inhibited (Fig. 4*D*). Phosphorylation of LHC2 is a mechanism underlying the state transition in higher plants (Allen *et al.* 1981, Rintamäki *et al.* 1997, Carlberg *et al.* 1999). Recently, a novel thylakoid-associated protein kinase, Stt7, was reported to be required for LHC2 protein phosphorylation and for state transition in *Chlamydomonas* sp. (Depege *et al.* 2003). Experiments have proved that the activity of phosphorylation kinase and phosphorylation of LHC2 can be strikingly suppressed by DTT (Rintamäki *et al.* 2000, Depege *et al.* 2003). Therefore, we suggest that the inhibition of phosphorylation of LHC2 might restrain the state transition, which might further exacerbate the shift in the balance of excitation energy distribution between PS1 and PS2.

In this study, the photon-saturated-photosynthetic rate was really depressed in the presence of DTT, though the photochemical efficiency of open PS2 centres increased (Figs. 2 and 3*A*). Obviously, the decrease of photosynthetic rate in the DTT treated leaves was not caused by inactivation of PS2, as indicated by the dark-recovery courses of F_v/F_m (Fig. 1). A serious shift in the balance of excitation energy distribution between PS2 and PS1 in the DTT treated leaves was accompanied by evident decrease of photosynthesis (Figs. 3*C,D* and 4*A*), indicating that to keep high utilization efficiency of absorbed photon energy, it might be necessary to maintain the balance of excitation distribution between PS2 and PS1. Külheim *et al.* (2002) reported that the fitness of two *Arabidopsis thaliana* mutants (*npq1* and *npq4*) that lack the essential proteins for feedback de-excitation was greatly reduced under field conditions. He argued that the reduction in fitness of the two *A. thaliana* mutants might be due to the decrease of photosynthesis (Külheim *et al.* 2002). Simultaneously, Li *et al.* (2002) observed that apparent quantum yield of oxygen evolution in an *npq4* mutant was distinctly decreased in parallel with the increase of excitation pressure of PS2. Therefore, we deduce that the shift in the balance of excitation distribution between PS2 and PS1 might lead to the decrease of photosynthesis. However, the inhibition of photosynthesis by DTT might be partially due to the interference with enzyme activation in the Calvin cycle or other physiological processes. We could not completely leave this reason out.

In conclusion, we think that the shift in balance of excitation energy distribution between PS1 and PS2 induced by feeding DTT was mainly due to the inhibition of state transition and xanthophyll cycle in leaves. It might be the shift in the balance of excitation energy between the photosystems that is mainly responsible for the decrease of photosynthesis.

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