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Phosphorylation/dephosphorylation of D1 protein alone has no effect on the electron transport activity of photosystem II in soybean leaves

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Introduction

In response to high light, plants can protect themselves from photodamage by several kinds of heat-dissipation of excess excitation energy, of which the xanthophyll cycle-dependent energy dissipation has been intensively studied (Demmig-Adams and Adams 1996). However, energy dissipation dependent on reversible inactivation of PSII reaction centers is still an unclear but attractive hypothesis (Chow 1994). Recently, it was demonstrated that photoinactivated PSII complexes could protect the functional neighbours (Lee et al 2001).

We have found that the predominant mechanism of energy dissipation in some plants such as soybean and cotton is dependent on the reversible inactivation of some PSII reaction centers (Hong and Xu 1999a). The reversible inactivation is related to the dissociation of LHCII from PSII complex and phosphorylation/dephosphorylation of LHCII is involved in its dissociation and recombination (Hong and Xu 1999b). However, one thing that is not clear yet is whether the reversible inactivation of PSII reaction centers is related to phosphorylation of D1 protein. Moreover, there are some different results about the relationship between the phosphorylation of D1 protein and the function of PSII (Harrison and Allen 1991, Aro et al 1992). The result presented in this report show that phosphorylation of D1 protein alone has no significant effect on the electron transport activity of PS II in soybean leaves.

1.Material and Methods

1.1 Soybean plants were grown in plots in the field. The fully expanded leaves were used in the experiments.

1.2 The petioles of two opposite leaflets were cut from a trifoliolate leaf under water. The petiole of one was put in water as control and the petiole of the other in a small Eppendorf tube containing 1 mM FSBA solution. After dark-adaptation for 3 h, these leaves were illuminated with weak light of 100 μmol photons m^{-2} s^{-1} to promote the transport of FSBA into leaves by transpiration stream.

1.3 The phosphorylated (D1*) and non-phosphorylated (D1) of D1 protein were detected by Western Blotting. The immunoblots were scanned with Gel-Doc laser densitometer to determine their relative amounts of proteins.

1.4 The electron transport activity of PSII was measured according to Zhang et al. (1988) with some modifications.

1.5 The measurement of chlorophyll fluorescence parameters was performed as described previously Hong and Xu 1999a .

2. Results and Discussion

2.1. Effect of FSBA treatment on phosphorylation level of D1 protein

Immunoblots of thylakoid membrane proteins from soybean leaves showed that there were two forms of D1 protein, phosphorylated (D1*) and non-phosphorylated D1 protein (D1). The percentage of D1* could be up to 74% in total D1 proteins in soybean leaves after dark-adaptation for 3 h. After treatment with 1mM FSBA, moreover, D1* could be dephosphorylated, leading to transformation of D1* to D1 (Fig.1 A). Fig.1 B is a qualitative demonstration of D1* and D1 in Fig.1 A scanned by Gel-Doc laser densitometer. After FSBA treatment for 1 h, the ratio of D1* to D1 decreased significantly and D1* completely disappeared after treatment for 2 h. Perhaps the high percentage of D1* in dark-adapted soybean leaves has two sides of physiological significance. On the one hand, an accumulation of D1* by a substantial amount may prevent its degradation and net loss, and thus provides a suitable base for the reversible inactivation of PSII reaction centers under high light illumination (Hong and Xu 1999a). On the other hand, both D1 proteins and LHCII need to be phosphorylated under high light. Although their phosphorylations are linked with different enzyme systems (Bennett 1991), there is a competition between them for ATP. So the substantial accumulation of D1* can decrease the competition for ATP between D1 protein and LHCII. Thus, there will be enough ATP for LHCII phosphorylation and thereby its dissociation from PSII reaction centers complex, which is an important step for the reversible inactivation of PSII (Hong and Xu 1999b).

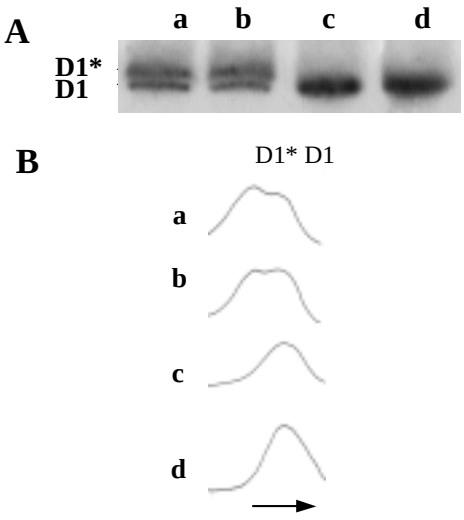

Fig. 1. The effect of FSBA treatment on the phosphorylation level of D1 proteins in soybean leaves.
[A] Western Blotting; [B] Qualitative analysis of D1* and D1 with a Gel-Doc laser densitometer. The arrow shows the direction of protein mobility. (a) control; (b), (c) and (d) represent FSBA (1mM) treatment for 1, 2 and 3 h, respectively.

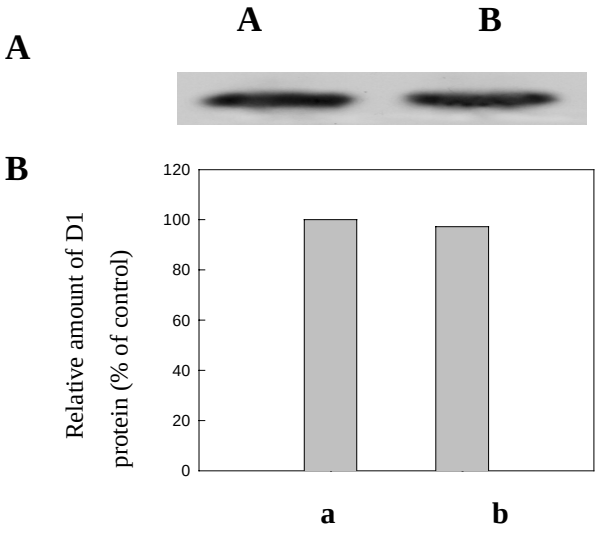

Fig. 2. The effect of FSBA treatment on the amount of D1 proteins in soybean leaves
[A] Western Blotting; [B] Quantitative demonstration of D1 proteins with a Gel-Doc laser densitometer. (a) control; (b) FSBA (1mM) treatment

2.2. Effect of FSBA treatment on the amount of D1 protein

From Fig. 2, it can be seen that there is a slight change in D1 protein amount (about 97% of control) after FSBA treatment for 3 h. The result indicates that although all phosphorylated D1 proteins (about 74% of total D1 proteins) have been transformed into non-phosphorylated form, no substantial loss of D1 proteins occurs.

2.3 Effect of FSBA treatment on chlorophyll fluorescence parameters

Fo and Fv/Fm were not significantly changed by FSBA treatment in soybean leaves (Table 1). After the FSBA treatment for 3 h, all of D1* have been completely dephosphorylated, but Fo and Fv/Fm decreased by about 4% and 1%, respectively.

Table 1 Effect of FSBA treatment on the chlorophyll fluorescence parameters in soybean leaves. Each value in the table is the mean of 3 leaves with standard error, and the values in the parentheses are the percentage of control.

		Fo	Fv/Fm
Control		0.152 ± 0.003 (100)	0.850 ± 0.002 (100)
FSBA	1 h	0.152 ± 0.001 (100)	0.838 ± 0.004 (99.3)
treatment	2 h	0.148 ± 0.003 (97.6)	0.839 ± 0.004 (99.2)
(1 mM)	3 h	0.145 ± 0.004 (95.7)	0.835 ± 0.001 (98.8)

The increase in Fo has been taken as a sign of damage of PSII reaction centers, mainly due to the loss of D1 protein (Franklin et al 1992), or inactivation of some PSII reaction centers (Critchley and Rusell 1994), while a decrease in Fo shows an enhancement of heat dissipation of the excitation energy (Demmig et al 1987). Therefore, the almost unchanged Fo and Fv/Fm in our experiment may indicate that there is no damage or inactivation of the PSII reaction centers after dephosphorylation of D1* caused by FSBA treatment.

2.4 Effect of FSBA treatment on photosynthetic electron transport activity of PSII

FSBA treatment had no effect on the light-saturated electron transport activity of PSII ($H_2O \rightarrow$ 1,4-BQ), even though all of D1* have been dephosphorylated completely after FSBA treatment for 2 h, as shown in Table 2.

Table 2. Effect of FSBA treatment on the photosynthetic electron transport activity of PSII ($H_2O \rightarrow$ 1,4-BQ) in soybean chloroplasts. The activity was measured at saturating light (1200 μmol photons m^{-2} s^{-1}), and the activities of the control were 100-200 μmol O_2 mg^{-1} Chl h^{-1}. The values in the table are the percentages of the control.

	1	2	3	4	Mean
FSBA treatment (1mM, 2h)	111.0	100.0	99.5	106.2	104.2 ± 2.8

In the past, most studies on D1 protein phosphorylation were focused on its function of protecting D1 protein from degradation and thereby disassembly of PSII complex. With respect to the effect of D1 phosphorylation itself on the function of PSII, there were different results and viewpoints (Harrison and Allen 1991, Aro et al 1992). We guess that whether D1 phosphorylation affects the structure and function of PSII may be dependent on illumination conditions. In the absence of high light the phosphorylation of D1 itself may have no

remarkable effect on either structure or function of PSII reaction centers. Therefore, neither structure nor function of PSII reaction centers is changed significantly by the dephosphorylation of D1*. The following facts reported here support this guess. (1) No significant increase in Fo and decrease in Fv/Fm, which are often considered the indication of damage or inactivation of PSII reaction centers, were observed after complete dephosphorylation of all D1* (Table 1). (2) After dephosphorylation of the D1* (about 74% of total D1 proteins), the D1 protein amount decreased by about 3%, which is negligible as compared with 74% (Fig. 2). This decrease may be the result of the low light syndrome (Ohad 2000) occurring under low light when treated with FSBA. (3) The dephosphorylation of all D1* did not cause a significant change in PSII electron transport activity (Table 2). We conclude that D1 protein phosphorylation/de phosphorylation alone has no significant effect on the function of PS II in soybean leaves.

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