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Complex regulation of Photosystem II protein phosphorylation via redox state of chloroplast

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Introduction

Redox state of chloroplast regulates the reversible phosphorylation of four PSII core proteins (D1 and D2 reaction centre proteins, CP 43 chlorophyll *a*-binding protein and psbH gene product) and three chlorophyll *a/b*-binding proteins of PSII; Lhcb1 and Lhcb2 (designated as LHCII) as well as Lhcb4 proteins. This redox control is mediated via regulation of the activation/activity of the protein kinases in thylakoid membranes. At the present, three chloroplast redox compounds are known to be involved in the regulation of reversible phosphorylation of PSII proteins: plastoquinone and cytochrome b_6f complex in thylakoid membranes (Vener et al. 1997) and a redox component with reactive thiols (Rintamäki et al. 1997, 2000). Alteration of incident light intensity in plants is a simple system to manipulate reduction level of these components and accordingly the amount of phosphorylated PSII proteins in chloroplast. Recently the general occurrence of the regulation mechanism for PSII protein phosphorylation has been challenged. The phosphorylation level of PSII core proteins in *Arabidopsis thaliana* reacted only slightly to dark/light transition of plants (Vener et al. 2001). We have studied the regulation of PSII protein phosphorylation *in vivo* in several plant species including the model plant *Arabidopsis*. The chloroplast redox state was modified by changes in incident light intensity in the growth chamber. Furthermore, the recovery of LHCII protein phosphorylation after high-light-induced inactivation was studied in three plant species. In the present paper we show that there are at least two different regulation mechanisms of PSII protein phosphorylation, one for PSII core proteins and another for LHCII proteins, and the mechanisms are universal in higher plants.

Materials and methods

Arabidopsis thaliana ecotype Wasellewskija was grown in the growth chamber with an 8-h photoperiod. The light/dark regime in growth chamber mimicked the natural light conditions: 10 and 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for first and second hours of light period, respectively, followed by the illumination for four hours at 230 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The gradual decrease of light intensity was applied at the end of the light period. Leaf samples were taken from plants at different times of light/dark regime as indicated in Fig. 1. Illumination of pumpkin and spinach leaves at a PFD of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for two hours and the isolation of thylakoid membranes were performed as described earlier (Rintamäki et al. 2000). *In vitro* phosphorylation of PSII proteins in isolated thylakoids was carried out as described in Rintamäki et al. (2000). The phosphorylation level of thylakoid PSII proteins was immunodetected with commercial polyclonal phosphothreonine antibodies (Zymed and New England BioLabs) (Rintamäki et al. 2000).

Results

Phosphorylation of PSII proteins under diurnal dark/light regime

The phosphothreonine antibody from New England BioLabs cross-reacted strongly with phosphorylated CP43 and D2 proteins (P-D2 and P-CP43) as well as phosphorylated LHCII proteins (P-LHCII) but gave weaker signal with phosphorylated D1 protein (P-D1) (Fig. 1A). This cross-reactivity differs from that obtained with a phosphothreonine antibody from Zymed that recognized most strongly P-D1 (Rintamäki et al. 1997) and had a very weak cross-reaction with P-LHCII in *Arabidopsis* (data not shown).

We have earlier observed that the amount of PSII centers with phosphorylated core proteins varies *in vivo* in dark-adapted leaves of different plant species. A significant amount of D1 and D2 proteins, but especially that of CP43 proteins is in phosphorylated form in dark-adapted spinach (Rintamäki et al. 1997) and maize (Rintamäki and Aro 2001) leaves, while the phosphorylated forms of these proteins are in the minority in dark-adapted pumpkin leaves (Rintamäki et al. 1997). The core proteins were also strongly phosphorylated after a long dark period in *Arabidopsis* leaves (Fig. 1A, lane 1). Illumination of *Arabidopsis* plants at very dim light induced dephosphorylation of these dark-phosphorylated proteins (Fig. 1A, lane 2), whereas the amount of PSII centers with phosphorylated core proteins increased again with increase in the irradiance at growth chamber (Fig. 1A, lanes 3 and 4). The decrease in light intensity in the end of the light period again induced dephosphorylation of PSII core proteins (Fig. 1A, lanes 5 and 6): a lowest amount of PSII centers with phosphorylated PSII core proteins occurred at very dim light in the end of the light period (Fig. 1A, lane 6). Interestingly, the amount of phosphorylated core proteins, especially that of P-D2 and P-CP43, increased again rapidly in darkness after the light period (Fig. 1A, lane 7).

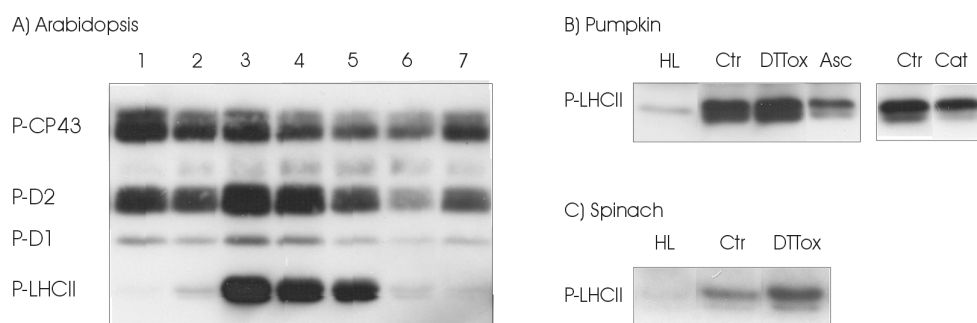


Fig. 1. A) Phosphorylation level of PSII proteins in *Arabidopsis* leaves during the diurnal light/dark regime. Leaf samples were collected at following times of the dark/light regime (see Materials and methods): 1) 15-h dark adaptation; 2) 1-h illumination at $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 3) 1-h illumination at $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 4) 4-h illumination at $230 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 5) 1-h illumination at $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 6) 1-h illumination at $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; 7) 2-h dark adaptation.

B and C) Effect of a thiol oxidant and scavengers of reactive oxygen species on the recovery of LHCII protein phosphorylation *in vitro*. Thylakoid membranes were isolated from high-light-illuminated pumpkin (B) and spinach (C) leaves. *In vitro* phosphorylation of thylakoids was carried out in light for 20 min in the absence (Ctr) or presence of 2 mM oxidized dithiothreitol (DTTox), 10 mM ascorbic acid (Asc) or catalase (200 U/ μg chlorophyll) (Cat). Phosphorylation level of LHCII proteins in thylakoid membranes prior *in vitro* phosphorylation (HL) is also presented in the figure.

LHCII phosphorylation in *Arabidopsis* during diurnal dark/light regime differed from that of core proteins. Long dark-adaptation of plants did not induce any significant phosphorylation of LHCII proteins (Fig. 1A). Generally, the phosphorylation of these proteins during light period followed the trend earlier observed for other higher plants (Rintamäki et al. 1997, 2000, 2001) with highest phosphorylation level in low light and a gradual decrease in phosphorylation at higher light intensities.

Recovery of LHCII phosphorylation in vitro after inactivation in vivo

High-light illumination induces inactivation of LHCII phosphorylation, that restores very slowly in low light *in vivo* (Rintamäki et al. 1997). The recovery process *in vitro* in isolated thylakoid membranes varied in different plant species. Restoration of the ability to phosphorylate LHCII proteins in isolated spinach thylakoids required the addition of a thiol oxidant (Fig. 1C). On the other hand, only short incubation of thylakoids isolated from high-light-illuminated pumpkin (Fig. 1B) and *Arabidopsis* (data not shown) leaves restored LHCII phosphorylation without addition of any oxidants. However, this recovery was inhibited in the presence of catalase (Fig. 1B), and also if ascorbic acid was present during *in vitro* phosphorylation of isolated thylakoids in light (Fig. 1B) but not in darkness (data not shown). Such ascorbate-induced inhibition of LHCII phosphorylation is unique for thylakoids isolated from high-light-illuminated leaves, since no inhibition occurred in thylakoids isolated from dark-adapted pumpkin leaves (Rintamäki et al. 2000). The experiments presented in Fig. 1B and C indicate that the restoration of phosphorylation activity for LHCII proteins requires an oxidant that is present in thylakoid preparation isolated from high-light-illuminated pumpkin and *Arabidopsis* leaves but is missing in preparations of spinach leaves.

Discussion

We conclude that phosphorylation of PSII proteins is under the complex control of chloroplast redox state that depends both on abiotic factors and on internal metabolic state of leaves that varies in different plant species. Phosphorylation level of PSII core proteins depends on the reduction of plastoquinone pool (Rintamäki et al. 2000) and reflects the chloroplast metabolic state: the plants having high phosphorylation level of PSII core proteins in dark-adapted leaves most probably keep the plastoquinone pool at least partially reduced in darkness, e.g. via chlororespiration. The components of the chlororespiration metabolism have recently been characterized in more detail (Nixon 2000). It is shown in Fig. 1A that the amount of PSII centers with phosphorylated core proteins is lowest in very dim light. This may be caused by efficient oxidation of plastoquinone pool at very low light and consequent inactivation of protein kinase involved in phosphorylation of PSII core proteins. Furthermore, it has become clear that restoration of LHCII phosphorylation after high-light-induced inactivation requires an oxidant that is able to oxidize thiols in proteins (Fig. 1B and C, Carlberg et al. 1999; Rintamäki et al. 2000). The availability of this oxidant seems to vary in chloroplasts of different plant species under various physiological conditions. Maximal phosphorylation of LHCII proteins *in vitro* occurs in spinach thylakoids isolated from low-light-illuminated leaves, while the capability to phosphorylate LHCII proteins in thylakoids isolated either from dark-adapted or high-light-illuminated spinach leaves increases significantly in the presence of a thiol oxidant (Fig. 1C, Carlberg et al. 1999). Induction of maximal LHCII phosphorylation in thylakoids isolated from dark-adapted pumpkin leaves, on the contrary, does not require any oxidant (Rintamäki et al. 2000). Moreover, the thylakoid membranes isolated from high-light-illuminated pumpkin contained an oxidant that restored the capacity of LHCII phosphorylation during short incubation of thylakoids *in vitro*. This

oxidant, however, was abolished by scavengers of reactive oxygen species, like catalase and ascorbate (Fig.1B).

Acknowledgements

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