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Illumination modulates the accessibility of the light-harvesting II complex (LHCII) phosphorylation site to the protein kinase

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Introduction

The state transition process is regulated via the reversible phosphorylation of the LHCII complex. Reduction of plastoquinone and cytochrome *bf* complex are involved in this process (Gal et al., 1997; Vener et al., 1997; Zito et al., 1999). According to this scheme one would expect that LHCII phosphorylation *in situ* would reach a maximal level at light intensities saturating electron flow. However, phosphorylation is drastically reduced in leaves exposed to high light intensities (Rintamäki et al., 1997; Vener et al., 1998), possibly via dithiols redox regulation (Carlberg et al., 1999; Rintamäki et al., 2000) We have recently demonstrated that illumination of isolated LHCII induces reversible exposure of the phosphorylation site at the N-terminal domain to protein kinase(s) in an *in vitro* reconstituted system (Substrate activation, Zer et al., 1999). A similar situation occurs for CP43 in isolated PSII-core complexes (Vink et al., 2000)**.** Here we report that illumination of thylakoids under conditions preventing LHCII phosphorylation induces further changes in the LHCII organization lowering its accessibility to the protein kinase. This phenomenon may contribute to inhibition of LHCII phosphorylation by high light intensities seen under physiological conditions.

Materials and Methods

Pea (*Pisum sativum*) was grown and preparation of isolated LHCII, thylakoids and phosphorylation procedures was as described (Zer et al., 1999). The activation of the protein kinase during thylakoid illumination was prevented by the addition of DCMU (10 μM) and was activated by addition of 1 mM duroquinol during the subsequent phosphorylation in darkness for 20 min using ^{32}P-γ-ATP followed by SDS-PAGE and autoradiography. Isolated LHCII was phosphorylated by solubilized thylakoid protein kinase (PI-K) (Zer et al., 1999). Detached pea leaves floating on water were exposed to 900 μmol photons m^{-2} s^{-1}. Tryptic digestion was as described (Zer et al., 1999*).*

Results

Effect of thylakoid illumination on the exposure of LHCII to protein kinase

Pre-illumination of thylakoids in the presence of DCMU followed by phosphorylation in darkness exhibited a transient activation of the LHCII substrate, increasing its phosphorylation level with the pre-illumination time. However, phosphorylation decreased with increase in the pre-illumination time and light intensity (**Fig. 1**). Since the thylakoid protein kinase remained active (**Fig. 2**), the light induced inaccessibility of the N-terminal domain of LHCII containing the phosphothreonine site to the protein kinase may be due to lateral aggregation of LHCII/PSII (Horton et al., 1994).

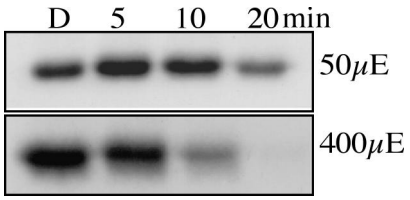

Fig. 1. Effect of pre-illumination on thylakoids LHCII phosphorylation
Autoradiogram showing the phosphorylation of LHCII in thylakoids in darkness subsequent to illumination in the presence of DCMU.

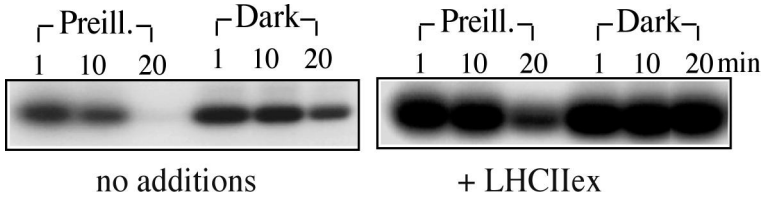

Fig. 2. Pre-illumination of thylakoids does not inactivate the protein kinase
Thylakoids were pre-incubated in the light (80 μmol photons m^{-2} s^{-1}) or in darkness for times as indicated followed by phosphorylation with ^{32}P-γ-ATP in darkness for 20 min. Isolated, exogenous LHCII (LHCIIex) was added during the pre-illumination period, (Preill.).

The light-induced inaccessibility of LHCII to the protein kinase decreases by lowering the temperature (**Fig. 3**). Preliminary results indicate that this effect is induced primarily by light absorbed by chl b (644 nm) despite the lower absorption by chl b as compared to chl a in both isolated and thylakoid bound LHCII (**Fig. 4**). However, illumination does not induce the occlusion of the phosphorylation sites to the protein kinase in isolated LHCII in an *in vitro* reconstituted system (Zer et al., 1999). Thus, the effect observed *in situ* may be due to light-induced lateral aggregation of the complex preventing the access of the kinase to the phosphorylation site. This conclusion is supported by results demonstrating that pre-illumination of thylakoids in presence of DCMU lowers the exposure of the membrane bound LHCII N-terminal domain also to trypsin cleavage (not shown) while increasing its exposure in isolated LHCII (Zer et al., 1999).

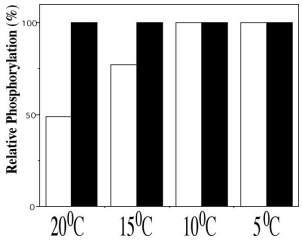

Fig. 3. Temperature dependence of the light-induced inaccessibility of LHCII to the protein kinase Thylakoids were preilluminated in the presence of DCMU for 20 min. at temperatures as indicated. The membranes were then phosphorylated in darkness for 20 min at 25 ºC.

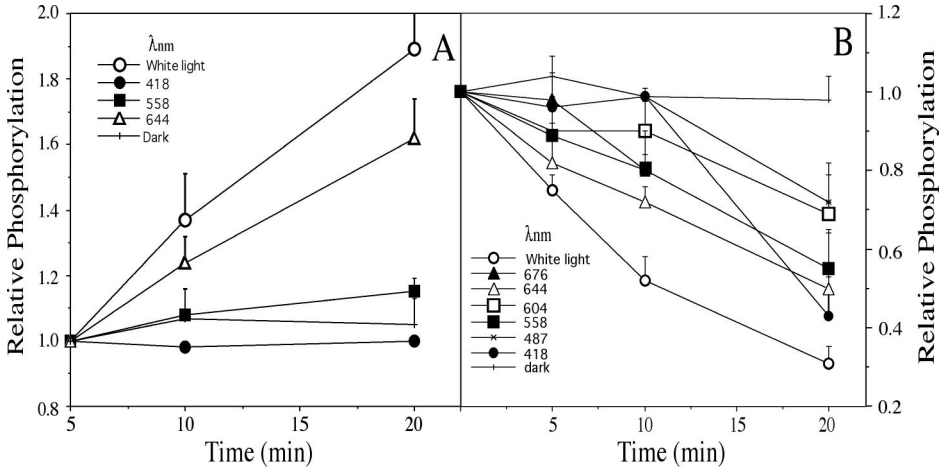

Fig. 4. Effect of pre-illumination time and light quality on the phosphorylation of LHCII in darkness Isolated LHCII or thylakoids were preilluminated (200 µg chl ml^{-1}; 80 µmol photons m^{-2} s $^{-1}$ at the sample level) for indicated times with white light or light filtered by interference filters of different wavelengths. The samples were then phosphorylated in darkness for 20 min followed by SDS-PAGE, autoradiography and quantification of the phosphorylation extent by scanning; A, isolated LHCII phosphorylated by the solubilized PI-K enzyme; B, thylakoids preilluminated in presence of DCMU; Note that the above illumination intensity enhances phosphorylation of isolated LHCII (substrate activation, Zer et al., 1999).

Effect of illumination *in vivo* on the exposure of the LHCII N-terminal domain to the protein kinase Illumination of detached leaves under conditions preventing the protein kinase activation by addition of DCMU, lowers the accessibility of LHCII to subsequent phosphorylation in darkness. (**Fig. 5**).

Discussion

The light-induced inaccessibility of LHCII to the protein kinase *in situ* was detected under conditions preventing phosphorylation during the illumination period. This phenomenon represents an additional, previously unexpected property of the chl-protein phosphorylation process that may play an important role in the regulation of the pigment-protein complexes interaction *in vivo*. The process occurs in two steps, "substrate activation" via conformational change of the N-terminal domain of LHCII (this work and Zer et al., 1999) followed by aggregation of the "light activated" complex in *situ* preventing access of the protein kinase to LHCII.

The phosphorylation/dephosphorylation of LHCII *in vivo* is a continuous process. Thus, a part of the mobile LHCII population bound to PSII is not yet phosphorylated, a part

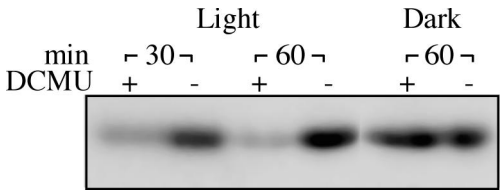

Fig. 5. Light induced inaccessibility of LHCII to the protein kinase *in vivo* Detached pea leaves maintained in constant humidity in the absence or presence of DCMU were exposed to 900 μmol photons m^{-2} s^{-1} or incubated in darkness for times as indicated. At the end of the incubation, thylakoids were prepared in dim green light and phosphorylated in darkness with ^{32}P-γ-ATP and activating the protein kinase by addition of duroquinol. The thylakoid proteins were resolved by SDS-PAGE followed by autoradiography.

has been phosphorylated, detached from PSII and bound to PSI and another part is already dephosphorylated and dissociated from PSI. Illumination of the non-phosphorylated LHCII population may induce lateral aggregation preventing the access of the exposed N-terminal domain to the protein kinase. This population may increase with the increase in the light intensity and phosphate turnover at the LHCII phosphothreonine site. Thus, phosphorylation maintains LHCII as phospho-LHCII in a "free" form minimizing the tendency of light exposed membrane-bound LHCII complexes to aggregate during the process of state transition.

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