S4-020

Light-harvesting complex II kinase and chloroplast redox signaling

S Pursiheimo, CH Hou, E Rintamäki and EM Aro

*Department of Biology, University of Turku, FIN-20014 Turku, Finland, Fax: +358-2-333 8075, E-mail:*evaaro@utu.fi

Keywords: Light-harvesting complex II, phosphorylation, Lhcb gene expression, PSII excitation pressure, redox regulation,

Introduction

Mature leaves adjust the composition of the photosynthetic machinery in response to various environmental stimuli. Acclimation of mature chloroplasts to changed environmental conditions is at least partially self-regulating, and involves redox signals that modulate the expression of photosynthesis related genes of both the chloroplast and nuclear genomes (Anderson et al. 1995). Regulation of the nuclear genes encoding light-harvesting complex II (LHCII) polypeptides has been under particular interest. Increased reduction of the plastoquinone pool in chloroplasts has been suggested to be coupled to a repression of *Lhcb* gene transcription in the nucleus, eventually leading to a decrease in the LHCII antenna size (Escoubas et al. 1995). Transcription of *Lhcb* genes is also under feed-back regulation by metabolic signals with strong transcriptional repression upon accumulation of soluble carbohydrates in cells (Graham et al. 1996).

Another LHCII-related process, strongly regulated by chloroplast redox signals is the phosphorylation of LHCII proteins. Reversible light-dependent phosphorylation of the two major polypeptides of the LHCII complex is under complex regulation (see Rintamäki and Aro 2001). Activation of the kinase responsible for LHCII protein phosphorylation requires a reduction of plastoquinone (Bennett 1991, Gal et al. 1997) and its binding to the Qo site of the cyt b_6/f complex (Vener et al. 1997). At high light intensities, however, a second loop of redox regulation is activated and LHCII phosphorylation becomes down-regulated via inhibition of LHCII kinase by thiol reductants (Rintamäki et al. 2000). Reversible LHCII protein phosphorylation has been discussed in the context of short-term regulation of light harvesting capacity via state transitions (Allen et al. 1992). We asked whether LHCII protein phosphorylation, or the respective kinase, is involved also in long-term adjustments of the LHCII antenna size. To this end, we explored relationships and common components involved in LHCII protein phosphorylation and in the expression of *Lhcb* genes.

Materials and methods

Control winter rye (*Secale cereale* cv. Voima) and pea (*Pisum sativum*) plants were grown at 300 µmol photons m^{-2} s^{-1} /20°C (300/20). Growth conditions of 50/20, 600/20 and 300/5 were applied when the effects of long-term acclimation under different conditions were studied. For short term acclimation studies the control plants were shifted from 300/20 to 50/ 20, 600/20 or 300/5 for 48 hours. A light/dark rhythm of 16/8 h was maintained during the experiments. To study the effect of exogenously added sugars, pea leaves were fed with glucose (250 mM) through petioles over night, and subsequently transferred to 50/20 or to PSII light (660 nm ± 20 nm, 30 µmol photons m^{-2} s^{-1}) for three hours. PSII excitation pressure ($1-Q_p$) was

monitored by measuring the photochemical quenching of chlorophyll fluorescence (Q_p) with a PAM fluorometer (Van Kooten and Snel 1990).

Leaves detached from plants after the treatment were immediately frozen in liquid nitrogen and thylakoids were rapidly isolated as previously described (Pursiheimo et al. 2001). Aliquots for isolation of RNA were taken from the initial leaf homogenate and immediately frozen in liquid nitrogen. After phenol extraction of RNA, the amount of *Lhcb* mRNA was studied by Northern blotting using barley *Lhcb* cDNA as a probe. To verify equal loading of RNA, the membranes were reprobed birch 18S rRNA gene. Thylakoid phosphoproteins were immunodetected with a commercial polyclonal phosphothreonine antibody (Zymed Laboratories Inc.) according to Rintamäki et al. 1997.

Results

A shift of winter rye plants from control growth conditions (300/20) to a 2-fold higher light intensity (600/20) or to low temperature (300/5) for 48 hours, induced an increase in the PSII excitation pressure, from 0.08 to 0.24. These shifts drastically decreased both the phosphorylation of LHCII proteins and the accumulation of *Lhcb* mRNA (Fig. 1). When plants were shifted from control growth conditions to lower light intensity (50/20), the excitation pressure of PSII declined to 0.03. This shift in light intensity was further reflected in an increase in both the phosphorylation state of the LHCII proteins and the accumulation of *Lhcb* mRNA.

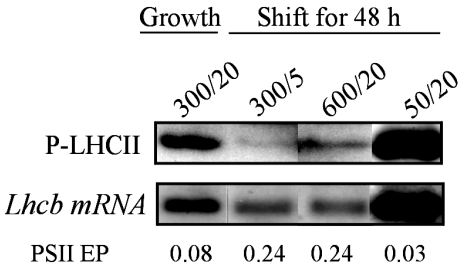

Fig. 1. Changes in LHCII protein phosphorylation (P-LHCII), PSII excitation pressure (EP) and accumulation of *Lhcb* mRNA upon short-term environmental shifts *in vivo*. Winter rye plants were shifted from control conditions (300/20) to different light/temperature regimes for 48 hours.

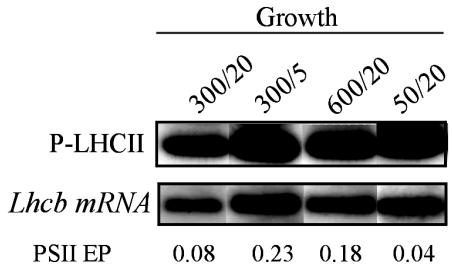

Fig. 2. Steady-state levels of LHCII protein phosphorylation (P-LHCII), PSII excitation pressure (EP) and the *Lhcb* mRNA content in winter rye plants after long term growth under different light and temperature regimes. Samples were collected four hours after the lights were turned on in each particular growth condition.

Long-term growth and acclimation of winter rye plants under contrasting light and/or temperature conditions modulated the steady state excitation pressures of PSII in rather similar extents as observed in short-term shifts to each particular condition (Fig. 2). In plants grown under 50/20, 300/20, 600/20 or 300/5, steady state PSII excitation pressures of 0.04, 0.08, 0.18 and 0.23 were recorded, respectively. Contrary to short-term experiments, and despite distinct differences in PSII excitation pressures, the LHCII polypeptides were always phosphorylated to some extent during the diurnal light phase. Noteworthy, also the high-light and low-temperature-grown plants accumulated *Lhcb* mRNA during the light period.

We also studied whether changes in sugar metabolism, which have been earlier shown to modulate *Lhcb* gene expression, are also reflected in the level of LHCII protein phosphorylation (Fig. 3). In control pea leaves incubated in water, only traces of LHCII

phosphorylation could be detected in darkness, whereas feeding of pea leaves with 250 mM glucose over night in darkness resulted in strong phosphorylation of LHCII. Low level of *Lhcb* mRNA was detected in both control and glucose-treated leaves in darkness. A sift of control plants to low light conditions for two hours induced a phosphorylation of LHCII and strong accumulation of *Lhcb* transcripts. On the contrary, a clear down-regulation of LHCII phosphorylation was observed in the glucose-treated leaves upon a shift to low light and this was accompanied by less distinct accumulation of *Lhcb* mRNA than in control leaves. The PSII excitation pressure, however, remained very low in both the control and glucose-treated leaves. Notably, when the control leaves or the glucose-treated leaves were illuminated under PSII light, strong phosphorylation of LHCII could be detected in both cases.

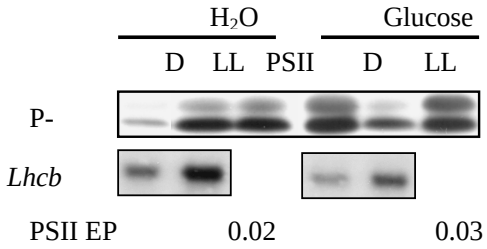

Fig. 3. Metabolic control of LHCII protein phosphorylation and *Lhcb* mRNA accumulation. Leaves were incubated in 250 mM glucose over night, and thereafter illuminated under low light or PSII light for two hours.

Discussion

Photosynthetic acclimation of mature plants involves a signal perception mechanism that is capable of sensing changes in the redox state of the chloroplast and of initiating a signalling cascade that modulates the expression of both the chloroplast and nuclear genes. To dissect components in the redox sensing pathway, we addressed the question whether the signaling cascade for regulation of *Lhcb* gene expression possibly shares components with the mechanism involved in reversible LHCII protein phosphorylation, also known to be strictly redox regulated (Rintamäki et al. 1997, 2000). Maximal phosphorylation of LHCII occures in low light when only a fraction of plastoquinone pool is reduced (Figs 1 and 3). However, with increase in PSII excitation pressure, clearly reduced steady-state levels of LHCII phosphorylation were detected (fig. 1). Importantly, however, such down-regulation of the LHCII kinase is mechanistically not connected to a high reduction state of the plastoquinone pool, as evidenced by maximal LHCII protein phosphorylation sustained by PSII light (Fig. 3). On the contrary, the redox components involved in inhibition of LHCII protein phosphorylation (i.e. the LHCII kinase) when plants are shifted to high light or low temperature originate from the reducing side of PSI, most likely being soluble stromal compounds like reduced thioredoxins (Rintamäki et al. 2000). It is conceivable that similar reduced compounds also accumulate in glucose-treated leaves and inhibit the LHCII kinase when leaves are illuminated.

We were particularly interested in dissecting whether the strict redox regulation of LHCII phosphorylation, and accordingly the activation state of the LHCII kinase, is reflected in the accumulation of *Lhcb* mRNA. Indeed, a clear coregulation between the capability for LHCII phosphorylation and for accumulation of *Lhcb* mRNA became evident during the 48-hour shifts in the light and/or temperature conditions (Fig. 1). Accumulation of *Lhcb* mRNA was induced under low light conditions that also maintained the LHCII kinase active. In sharp contrast, a shift of rye plants to higher irradiance or to low temperature largely abolished the phosphorylation of LHCII proteins and concomitantly induced dampening of the circadian accumulation of *Lhcb* mRNA (Fig. 1). Parallel down-regulation of LHCII protein

phosphorylation and *Lhcb* mRNA accumulation also occured when the sugar metabolism of the leaves was modulated (Fig. 2). Furthermore, the winter rye plants, upon long-term acclimation to high light or low temperature, gained abilities for both to maintain LHCII phosphorylation and to accumulate *Lhcb* mRNA under high excitation pressure of PSII.

Our results suggest a crucial role for the activation state of LHCII kinase, rather than the redox state of the plastoquinol pool, as an initiating factor in chloroplast redox signaling pathway to the nucleus. Such LHCII kinase model presupposes an induction of a transcriptional activator molecule in the nucleus, or alternatively a release of a repressor protein, as a response to an activation of the LHCII kinase in chloroplasts. Activity of the LHCII kinase, strictly controlled by environmental cues, would regulate the amount and/or the activity of such regulatory proteins and thereby the transcription of *Lhcb* genes, possibly via a cascade of phosphorylation/dephosphorylation reactions.

Acknowledgements

This work was financially supported by the Academy of Finland, the European community (ERB IC 15-CT98-0126) and Nordisk Kontaktorgan för Jordbruksforskning.

References

Allen JF (1992) *Biochimica Biophysica Acta* **1098**, 275-335

Anderson JM, Chow WS and Park YI (1995) *Photosynthesis Research* **46**, 129-139

Bennett J (1991) *Annual Reviev of Plant Physioloy and Plant Molecular Biology* **42**, 281-311

Escoubas JM, Lomas M, LaRoche J and Falkowski PG (1995) *Proceedings of the National Academy of Sciences USA* **92**, 10237-10241

Gal A, Zer H and Ohad I (1997) *Physiologia Plantarum* **100**, 869-885

Graham IA (1996) *Research in Microbiology* **147,** 572-580

Pursiheimo S, Mulo P, Rintamäki E and Aro EM (2001) *Plant Journal* in press

Rintamäki E, Salonen M, Suoranta UM, Carlberg I, Andersson B and Aro EM (1997) *Journal of Biological Chemistry* **272**, 30476-30482

Rintamäki E, Martinsuo P, Pursiheimo S and Aro EM (2000) *Proceedings of the National Academy of Sciences USA* **21**, 11644-11649

Rintamäki E and Aro EM (2001) in: Aro EM and Andersson B (eds.) Regulation of photosynthesis. Kluwer Academic Publishers, Dordrecht, *Advances in photosynthesis* **11**, 395-418.

Van Kooten O and Snel FH (1990) *Photosynthesis Research* **25**, 147-150.

Vener AV, van Kan PJM, Rich PR, Ohad I. and Andersson B (1997) *Proceedings of the National Academy of Sciences USA* **94**, 1585-1590.