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Comparison of plastoquinone reduction, LHCII phosphorylation and state transitions

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Introduction

Plants in their natural habitat are constantly exposed to transient or more long-term changes in the quantity and quality of light. In order to preserve a high quantum yield and protect themselves from photoinhibition they have evolved several mechanisms to regulate the absorption of excitation energy. One way to ensure a balanced flow of electrons between the two photosystems is by redistributing a mobile pool of light harvesting antenna complexes (LHCII). According to the accepted view, distribution of LHCII (state transitions) is regulated via the redox state of cytochrome b_6/f and the inter-chain electron carrier plastoquinone. When PSII receives more excitation energy than PSI (state 2) the plastoquinone pool becomes over-reduced and binding of plastoquinol to the Qo pocket of cytochrome b_6/f leads to the activation of one or several kinase(s) (Verner et al., 1995; Zito et al., 1999). The kinase phosphorylates LHCII, which dissociates from LHCII. Redistribution of photons to PSI at the expense of PSII will occur until the plastoquinone pool again becomes sufficiently oxidised. The kinase will then become inactivated and a phosphatase will dephosphorylate LHCII causing the movement of the mobile antenna back to PSII. A thylakoid-associated kinase, which phosphorylates LHCII under reducing conditions, has recently been identified (Snyders and Kohorn, 1999). When the kinase is down-regulated, LHCII is not phosphorylated and the plants do not exhibit state transitions (Snyders and Kohorn, 2001). Thus, state 1 reflects a low phosphorylation level of LHCII and state 2 a high. It has been suggested that the detachment of phosphorylated LHCII is caused by structural changes in the N-terminus, however, structural changes of LHCII are also seen upon light-treatment (Nilsson et al., 1997 and Zer et al., 1999).

Recently, a second regulatory level of LHCII phosphorylation was proposed involving the stromal ferredoxin-thioredoxin system. Rintamäki et al. (1997) observed that under high-light conditions the steady-state level of phosphorylated LHCII decreased and maximal levels were in fact seen under low-light conditions. Thus, the phosphorylation did not follow the plastoquinone reduction level, since plastoquinone is most highly reduced under high-light conditions. This effect was explained by the presence of a redox sensitive disulfide bond in the LHCII kinase; hence, reduction of the disulfide bond by reduced thioredoxin will inactivate the kinase (Rintamäki et al., 2000). In barley, however, state transitions are still occurring under high-light conditions (Quick and Stitt, 1989). Thus, the correlation between the redox level of the plastoquinone, LHCII phosphorylation and state transitions is not tight. We have characterised an *Arabidopsis thaliana* mutant lacking PSI-H, which is unable to perform state transitions (Lunde et al., 2000). Although the LHCII is highly phosphorylated it does apparently not dissociate from PSII. We therefore wanted to make a comparison of the

ability to perform state transitions, degree of LHCII phosphorylation and plastoquinone reduction level in *Arabidopsis thaliana*.

Materials and methods

The redox level of plastoquinone was determined by measuring the excitation pressure ($1-q_P$). q_P was calculated as $(F_m'-F_s)/(F_m'-F_0')$; where F_m' and F_0' is the maximal fluorescence induced by a saturating flash and the fluorescence of pre-illuminated leaves in the absence of actinic light, respectively. F_S is the steady state fluorescence (Haldrup et al, 1999).

State transitions during alternating blue and far-red treatments were measured as in Jensen et al. (2000). State transition measurements under different light intensities were done using white light instead of blue. The relative change in fluorescence was calculated as $F_r = ((F_i'-F_i)-(F_{ii}'-F_{ii}))/(F_i'-F_i)$; where F_{ii}' and F_i, is the steady state fluorescence in state 2 and 1, respectively. F_{ii} and $F_i'$is the fluorescence obtained upon exposing the leaf to PSI light in state 2 and to PSII light in state 1, respectively. F_{m1} and F_{m2} is the maximal fluorescence in state 1 and 2, respectively.

Levels of LHCII phosphorylation were determined by western blotting probing with phospho-threonine antibodies (Rintamäki et al., 1997). The leaves were treated for 20 minutes under the stated light condition except under growth light where the leaves were harvested after 4 h of light.

Results

When the intensity of the light and consequently the pressure on PSII is increased, the plastoquinone pool becomes more reduced (Table 1). Under the increasing white light from a Schott-lamp there is no significant difference in the plastoquinone redox level between wild-type and the state transition deficient plant (- PSI-H). In the PSI-H less plants, however, LHCII phosphorylation level is higher than wild-type at all light intensities. The phosphorylation level of LHCII in the wild-type peaks at 40 photons m^{-2} s^{-2} and decreases at higher light intensities (Fig. 1). A similar observation has been made with pumpkin plants and intact spinach chloroplast (Rintämaki et al, 1997 and Ebbert and Godde, 1994). Surprisingly, no major change was seen in the ability to perform state transitions. Between 40 and 80 photons m^{-2} s^{-2} the degree of state transition measured as F_{m1}/F_{m2} and F_r was unchanged and at 200 photons m^{-2} s^{-2} a minor decrease (10%) in relative state transitions was observed. Hence, a 50% decrease in LHCII phosphorylation is not causing a similar decrease in state transitions.

Table 1. The correlation between state transitions, redox level of plastoquinone and phosphorylation of LHCII at increasing light intensities. Leaves from dark-adapted plants were used for all three measurements. The determination of state transitions and q_P was done directly in a PAM101-102-103 fluorometer. For the LHCII-P measurements, the leaf was exposed for 20 minutes with the appropriate light.

Light intensity (photons m^{-2} s^{-2})	State transitions (F_{m1}/F_{m2}) **Wild-type**	Relative state transitions (F_r) **Wild-type**	Redox level of plasto-quinone pool ($1-q_P$) **Wild-type**	**-PSI-H**	Phosphorylation of LHCII **Wild-type**	**-PSI-H**
0	-	-	-	-	5%	80%
≈20	-	-	0.032±0.03	0.028±0.02	40%	140%
≈40	1.063±0.004	0.850±0.07	0.046±0.03	0.043±0.01	100%	170%
≈80	1.051±0.005	0.851±0.03	0.075±0.05	0.063±0.01	50%	200%
≈200	1.017±0.016	0.766±0.10	0.181±0.05	0.152±0.02	40%	140%

State transitions are normally measured by shifting the light between PSII light (blue) and PSI light (far-red). When leaves are exposed to PSI or PSII light the redox level of the plastoquinone pool becomes much more reduced in state 2 (Table 2). Under growth light conditions to which the plants are adapted only a minor reduction of plastoquinone is observed, however, the LHCII phosphorylation level is highest under growth light conditions in both the wild-type and the plants lacking PSI-H. Only a minor change in LHCII phosphorylation is seen between state 1 and 2 (Fig. 1).

Table 2. State transitions, redox level of plastoquinone pool and LHCII phosphorylation measured under different light conditions.

Light condition	State transitions (F_{m1}/F_{m2}) **Wild-type**	**- PSI-H**	Relative state transitions (F_r) **Wild-type – PSI-H**		Redox level of plasto-quinone pool ($1-q_P$) **Wild-type - PSI-H**		Phosphorylation of LHCII **Wild type - PSI-H**	
Dark	-	-	-	-	-	-	5%	80%
State 1	-	-	-	-	0.038±0.003	0.106±0.008	50%	150%
State 2	1.06±0.01	1.00±0.01	0.63±0.04	0.11±0.02	0.138±0.019	0.262±0.007	90%	150%
Growth light	-	-	-	-	0.009±0.002	0.083±0.004	100%	140%

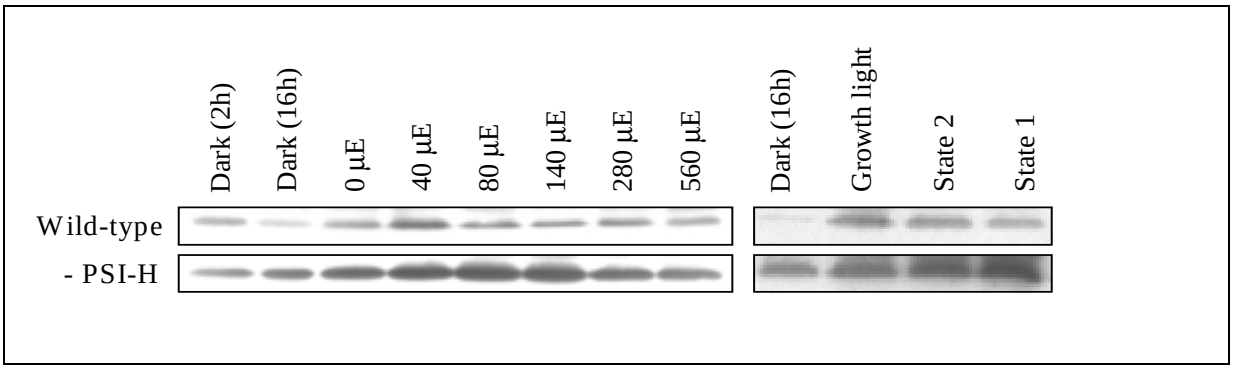

Figure 1. The phosphorylation of LHCII in wild type and plants lacking PSI-H exposed to varying light intensity and quality.

Discussion

A large number of experiments have shown that reducing conditions leads to kinase activity and that this kinase activity is a prerequisite for state transitions. However, since there is no simple correlation between plastoquinone reduction, LHCII phosphorylation and state transitions, we suggest that kinase activity does have a function in state transitions, but phosphorylation of LHCII might not be the key initiator of state transitions (Haldrup et al., 2001). Instead phosphorylation could play a protective role of dissociated LHCII and like e.g. phosphorylation of D1 act to stabilize the protein and prevent premature degradation (Koivuniemi et al, 1995). Protection from light induced degradation by phosphorylation of LHCII has been observed (Georgakopoulos and Argyroudi-Akoyunoglou, 1997). We have previously shown that binding of LHCII to PSI is necessary for its detachment from PSII, thus, state transitions could possibly be regulated via a alteration in the affinity for the two photosystems caused by a structural change (Allen, 1992). The pattern of LHCII phosphorylation, maximal at low light and minimal in darkness and under high light does not agree with a photoprotective role of LHCII phosphorylation, but it is also not clear what the physiological functions of state transitions are (Nyiogi, 1999; Lunde, unpublished results). Another suggested function of state transitions is to optimise the absorption of light by the two photosystems. In that respect it is surprising that the highest level of LHCII-phosphorylation apparently is seen under growth light conditions and not under PSII light and that steady-state phosphorylation is almost identical in state 1 and 2. Surprisingly, reversible phosphorylation of LHCII in leaves incubated with ^{33}P showed a ≈90% decrease in labelling upon changing the light from PSII to PSI light (Lunde et al., 2000). Apparently there is a sub-population of LHCII molecules which show fast and reversible phosphorylation in respect to light and another which is more or less constantly phosphorylated and even after 16h of darkness LHCII-P can be detected (Fig. 1).

We are currently characterising the PSI-H less plants in order to determine how the plants respond to the inability to perform state transitions and under which conditions state transitions are a selective advantage for the plant.

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