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Effect of cold temperature on regulation of state transitions in *Arabidopsis thaliana*

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ABSTRACT

Low temperature is one of the most important abiotic factors limiting growth, development and distribution of plants. The effect of cold temperature on phosphorylation and migration of LHCII has been studied by 77K fluorescence emission spectroscopy and immuno-blotting in *Arabidopsis thaliana*. It has been reported that the mechanism of state transitions has been well operated at optimum growth temperatures. In this study, exposure of leaves to cold conditions (10 °C for 180 min) along with low light treatment (for 3 h) did not show any increase in F726 which corresponds to fluorescence from PSI supercomplex, whereas low light at optimal temperature (26 ± 2 °C) could enhanced F726. Therefore these results conclude that low light at cold condition did not enhance PSI absorption cross-section. We have also observed low levels of LHCII phosphorylation in cold exposed leaves in dark or low light. Though LHCII phosphorylation was detectable, the lateral movement of phosphorylated LHCII is reduced due to high granal stacking in cold treated leaves either in light or dark. Apart from these results, it is suggested that increased OJ phase and decreased JI and IP phases of Chl *a* fluorescence transients were due to reduced electron transport processes in cold treated samples.

1. Introduction

Plants are continuously being exposed to different abiotic stress conditions particularly light and temperature during their growth. Cold temperatures are one of the abiotic stress factors which affect the growth and productivity [1]. Plants vary in their ability to withstand low temperature stress and there is much interspecific variation in the capacity for the regulation of photosynthetic metabolism and redox homeostasis at cold temperatures. Dark chilling is known to inhibit a wide range of physiological and metabolic processes in various plants [1,2]. Cold condition induced inhibition of photosynthetic performance in some plants, which is related to reduction of electron transport and metabolic restrictions [3]. The circadian regulation, enzyme activity and photosynthetic gene transcription were found to be impaired at cold temperatures [4–6]. It has been reported that cold temperature affects the membrane plasticity which is an important phenomena for mobilization of various hydrophobic proteins. Cold temperature reduces membrane fluidity which leads to reduced D1 turnover by inhibiting diffusion of photodamaged D1 proteins that has been marked for degradation from appressed region to non-appressed regions of the thylakoids [1]. Changes of temperature and light intensity influence the relative state of photosystem II (PSII) oxidation [7]. Such changes can modify the balance between energy utilization in the processes of growth and cold acclimation [8], as well as modifying the expression of some genes directly involved in cold acclimation [9].

Photosynthetic organisms are able to balance the light excitation energy between the two photosystems by a process called "state transitions" and thus adapting to environmental changes. State transitions were found to be an important for functional and structural regulation of energy distribution between photosystems for performing optimal photosynthesis in plants, green algae and cyanobacteria [10–12]. The molecular mechanism that is involved in state transitions for short term regulation of the absorbed energy distribution in algae and higher plants, is carried out by phosphorylation of light-harvesting chlorophyll *a*/*b*-protein complex (LHCII) [13,14]. A membrane bound kinase, STN7 kinase phosphorylate major LHCII of PSII [13,15]. The kinase activity is controlled by the redox state of electron transport components such as plastoquinone (PQ) and the cytochrome b_6/f (Cyt b_6/f) complex [12,16–18]. The redox state of the intersystem PQ pool in the linear electron transport chain acts as a signal for absorption imbalance between PSI and PSII [19]. In higher plants and green algae, the reduced state of PQ, along with the Cyt b_6/f complex, activates a thylakoid associated kinase called STN7/STT7 [18]. Phosphorylation of a specific light harvesting complex by STN7/STT7 kinase has been shown to be involved in the migration of light harvesting antennae from PSII to PSI, altering the relative

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absorption cross sections of both photosystems [10,20]. In state I, the PQ pool remains in the oxidized state, leading to high absorption and emission by PSII. Upon light-induced reduction of the PQ pool (state II), the STN7 kinase phosphorylates LHCII, resulting in migration of phosphorylated LHCII towards PSI, which is concentrated in the stromal lamellae of the thylakoid membranes. The migration of LHCII enhances the emission of PSI and simultaneously decreases the emission of PSII. When the PQ pool has been reoxidized by PSI, a thylakoid phosphatase designated as TAP38/PPH1 dephosphorylates LHCII, in turn decreasing its affinity for PSI and resulting in migration of LHCII back to the PSII rich grana regions [21,22]. This rearrangement in the organization of the light-harvesting antenna [14], requires a fluid membrane bilayer, and suggested to be important for balancing of excitation energy between the two photosystems. The mechanism of state transitions is not operational in rice subjected to chilling temperatures suggesting that protein phosphorylation might be have some role during protection against photoinhibition under chilling temperatures [23].

Movement of proteins along the plant thylakoid membrane is important in state transitions apart from turnover and repair of the PSII complex. Such lateral migrations are impaired at low temperatures, which could contribute to the increased sensitivity of plants to photoinhibitory damage at low temperatures. LHCII phosphorylation was identified to be strictly redox regulated under different abiotic stress conditions and plastoquinone was identified as a regulator of gene expression indicating that state transitions and changes in photosystem stoichiometry are operative simultaneously [24]. Mechanisms involved in the signaling events are likely to be initiated and disclosed directly after a shift in environmental conditions, and will thereafter gradually lead to acclimatization of plants to changed environment. The inhibition of photosynthesis by chilling is well established [25,26], however, relatively little is known about the state transitions mechanism under cold conditions. Low temperatures influence the phosphorylation of LHCII and the subsequent rearrangements in the organization of the thylakoid membrane. It was studied *in vitro* to understand the significance of lateral migration of a single protein component within the thylakoid membrane. It has been shown that the protein kinase is functional at low temperatures but that the rapid lateral migration of phospho-LHCII in the thylakoid membrane is largely prevented below 10 °C and there is no decrease in the antenna size of photosystem II [27].

In order to understand how cold temperature induces changes in the mechanism of state transitions, we made an attempt to study the changes in protein phosphorylation of LHCII in the thylakoid membrane during the initial hours of cold treatment in *Arabidopsis thaliana*. A change in LHCII phosphorylation was studied by immunoblotting analysis, migration of LHCII antenna and also energy distribution was performed using 77K fluorescene emission spectra. Also, we have monitored the effect of low temperature on electron transport by fast chlorophyll fluorescence transient analysis. Changes in LHCII phosphorylation during cold treatment along with high light and low light conditions would be discussed further.

2. Materials and methods

2.1. Growth condition

A. thaliana wt plants were grown in controlled environment chambers at 100–120 μmol m^{-2} s^{-1}, with 8 h light/16 h dark periods. Leaves were harvested from 6 to 7 week old plants.

2.2. Cold (10 °C) and low light treatments

The fully matured leaves were detached from wt plants and cold treated for different time periods from 0 to 180 min at 10 °C. Low light (LL) (35–40 μmol photons m^{-2} s^{-1}) or high light (HL) (1000 μmol photons m^{-2} s^{-1}) with or without cold. During the treatment the detached leaves were floated in a beaker containing water; temperature was maintained using an external water bath as described in earlier work [28]. Cold and/or light treated leaves were frozen in liquid nitrogen for immunoblotting and 77K fluorescence emission.

2.3. 77K Fluorescence emission measurements

Isolated thylakoid membranes were diluted in 60% glycerol containing thylakoid buffer with 10 μg Chl ml^{-1}. The emission spectra at 77K were measured in between 600 and 780 nm and excited wavelength is 436 nm by using Perkin Elmer, LS-55 fluorescence spectrophotometer. The emission spectra were recorded at 1 nm s^{-1}. Band width was 5 nm for both excitation and emission. The raw spectra were normalized at 685 nm for comparison of fluorescence emission bands emanating from PSI.

2.4. The fast OJIP fluorescence transient measurements

Chlorophyll fluorescence fast induction curves were measured using a plant efficiency analyzer (PEA), Hansatech, King' Lynn, Norfolk, UK. The dark adapted leaves were excited by an array of three light-emitting diodes peaking at 650 nm at a photon flux density of 3000 μmol photons m^{-2} s^{-1}. The fast fluorescence transients (OJIP) were measured [29] from cold induced temperature (10 °C) leaves of wt.

2.5. Immunoblotting of thylakoid membrane proteins

Tricine SDS–PAGE was carried out as described by Schägger and von Jagow [30]. For immunoblotting, proteins were separated by SDS–PAGE and were transferred onto polyvinyldenedifluoride (PVDF) membrane (Bio Rad) using a transblot apparatus (Bio Rad), according to the manufacturer's instructions. The blots were probed with rabbit anti-phosphothreonine polyclonal antibodies (1:2500 dilution) purchased from New England Biolabs (CST) to detect phosphorylated proteins at threonine site.

All experiments were repeated three times and the results were reproducible every time. Chlorophyll concentrations were determined spectroscopically according to Porra et al. [31].

3. Results and discussion

A. thaliana plants are normally freezing tolerant but become sensitive to chilling at 10–15 °C [32]. Light induced state transitions mechanism generally operate at low light under optimal growth (26 ± 2 °C) conditions. Recently we have reported that elevation of 15 °C (40 °C) from optimal growth temperature, enhanced dark reduction of PQ pool and LHCII phosphorylation [28,33] which mimics the light induced state transitions. In this study, we report the effect of cold temperature on mechanism of state transitions. When the temperature decreased to 10 °C from optimal growth temperature (26 ± 2 °C), the effect of 10 °C (cold) on mechanism of state transitions in *A. thaliana* was monitored by Chl *a* fluorescence, 77K fluorescence and immuno-blotting techniques. We also tested 5 and 15 °C, however, all the results were similar to 10 °C.