Journal of Plant Physiology 169 (2012) 65–71

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Strong light-induced reorganization of pigment–protein complexes of thylakoid membranes in rye (spectroscopic study)

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ARTICLE INFO

Article history:
Received 1 February 2011
Received in revised form 16 August 2011
Accepted 16 August 2011

Keywords:
LHCII aggregation
Light stress
Monomer
Photoprotection
Trimer

ABSTRACT

The supramolecular reorganization of LHCII complexes within the thylakoid membrane in *Secale cereale* leaves under low and high light condition was examined. Rye seedlings were germinated hydroponically in a climate chamber with a 16 h daylight photoperiod, photosynthetic photon flux density (PPFD) of 150 μmol m^{-2} s^{-1} and 24/16 °C day/night temperature. The influence of pre-illumination of the plants with high light intensity on the PSII antenna complexes was studied by comparison of the structure and function of the LHCII complexes and organization of thylakoid membranes isolated from 10-day-old plants illuminated with low (150 μmol m^{-2} s^{-1}) or high (1200 μmol m^{-2} s^{-1}) light intensity. Aggregated and trimeric with monomeric forms of LHCII complexes were separated from the whole thylakoid membranes using non-denaturing electrophoresis. Analyses of fluorescence emission spectra of these different LHCII forms showed that the monomer was the most effective aggregating antenna form. Moreover, photoprotection connected with LHCII aggregation was more effective upon LHCII monomers in comparison to trimer aggregation. Light stress induced specific organization of neighboring LHCII complexes, causing an increase in fluorescence yield of the long-wavelength bands (centered at 701 and 734 nm). The changes in the organization of the thylakoid membrane under light stress, observed by analysis of absorbance spectra obtained by Fourier transform infrared spectroscopy, also indicated light-induced LHCII aggregation.

Introduction

In the natural environment, the light condition of photosynthetic organisms can vary highly in intensity and quality on a broad time scale, ranging from minutes up to hours, days, and even months. The changes resulting from cloud movement, sun flecks, weather system, sudden shading by other plants or changes in the canopy structure are frequently observed (Owens, 1994). The time of exposure of plants to the light of high intensity can amount to even a few hours daily. Short- as well as long-term illumination of plants with high light frequently leads to photoinhibition of the photosystem II (PSII) complex, which is connected with photodamage of the D1 polypeptide, photosynthetic pigments and unsaturated fatty acids of thylakoid membranes (Aro et al., 2004; Edelman and Mattoo, 2008; Tyystjärvi, 2008). Because of rapid and irregular fluctuations of light intensity, plants have developed several regulatory photoprotective mechanisms operating at different levels of their organization. A very effective type of adaptation of the photosynthetic apparatus to high light illumination occurs at the molecular level. It takes place within the thylakoid membrane, which is a dynamic system regulating the efficiency of absorption, utilization and dissipation of light energy. This system is characterized by a hierarchical arrangement of pigment protein complexes of photosystem II, photosystem I and its light-harvesting complexes (LHC). Dimeric and monomeric forms of PSII occur in the stacked and stromal thylakoid membranes, respectively (Dekker and Boekema, 2005). The largest PSII antenna complexes (LHCII) may associate with the dimeric PSII core yielding a LHCII–PSII supercomplex (Nield and Barber, 2006). The LHCII complexes which do not associate with PSII can oligomerize to forms having specific supramolecular organization (Ruban et al., 1997; Dekker et al., 1999; Holm et al., 2005; Gruszecki et al., 2009b). The native and functional form of LHCII is a trimer. Each monomer of the trimer is composed of a polypeptide of about 232 amino-acid residues constituting three transmembrane α-helices, named A, B and C, and also two short α-helices referred to as D and E. The polypeptide chain binds 8 molecules of chlorophyll (Chl) *a*, 6 molecules of Chl *b* and xanthophyll pigments such as one violaxanthin, one neoxanthin and two luteins (Liu et al., 2004). Under the light stress condition, the LHCII complex reversibly switches its function from light-harvesting into photoprotective dissipation

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doi:10.1016/j.jplph.2011.08.021

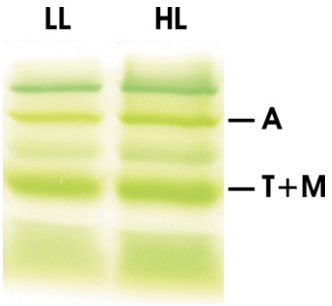

Fig. 1. The representative gel scan of Chl–protein complexes separated by native green gel electrophoresis. Thylakoid membranes were isolated from rye leaves pre-illuminated with low or high light intensity. Bands used for measurements are indicated in the figure. A – LHCII aggregates, T + M – LHCII trimers with monomers.

of absorbed energy. These changes in the LHCII function are connected with conformational rearrangements within the complex. One is enzymatic conversion of the antenna pigment – violaxanthin via anteraxanthin to zeaxanthin in the so-called xanthophyll cycle process (Sapozhnikov et al., 1957; Yamamoto et al., 1962). The major role of this interconversion is formation of energy traps, which effectively quench the excess excitation energy. Zeaxanthin can form a radical pair with a Chl molecule of LHCII (Holt et al., 2005) responsible for excess excitation energy dissipation. Other studies have shown that lutein molecules are required for efficient quenching of harmful Chl excited states (Dall'Osto et al., 2006). The physiological importance of the xanthophyll cycle is also attributed to formation of specific supramolecular organization of the LHCII complexes. This organization is characterized by aggregated LHCII forms with a regular ring-like structure (Gruszecki et al., 2009a). Very recently, Gruszecki et al. (2010a) showed that xanthophylls such as violaxanthin and, in particular, zeaxanthin, promote formation of quenching centers in LHCII complexes. Moreover, photoprotective energy dissipation results in singlet excitation quenching, which is regulated by blue light at the specific LHCII organization level (Gruszecki et al., 2010b).

To better understand the photoprotective mechanisms operating at the molecular level, we studied structural reorganization and functional changes of the whole thylakoid membranes and antenna complexes (LHCII) isolated from rye leaves illuminated with strong light.

Materials and methods

Plant material and growth conditions

Secale cereale L., cv Pastar seedlings were germinated in a climate chamber with a 16 h daylight photoperiod, photosynthetic photon flux density (PPFD) of 150 μmol m^{-2} s^{-1} and 24/16 °C day/night temperature (Janik et al., 2010). The seedlings were cultivated hydroponically (five plants per pot filled with 0.5 l of Hoagland nutrient solution). The nutrient solution was continuously aerated. After 10 days of cultivation, the plants were divided into two groups. One group was illuminated with 150 μmol m^{-2} s^{-1} (low light) and one with 1200 μmol m^{-2} s^{-1} (high light) for 3 h. Next, the first leaves of plants from both groups were harvested for measurements.

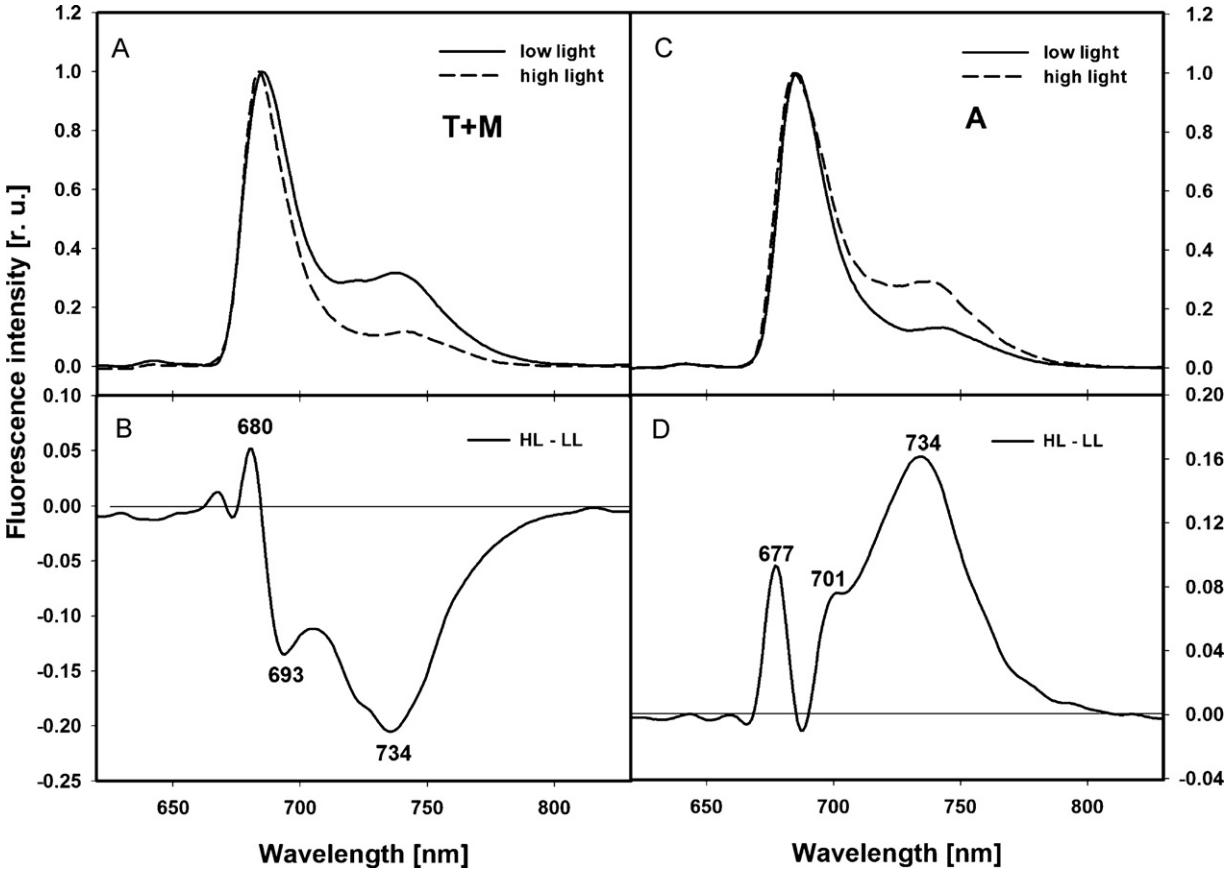

Fig. 2. 77 K Chl *a* fluorescence emission spectra of the trimeric with monomeric (T + M, panel A), and aggregated (A, panel C) forms of LHCII. The complexes were obtained by native green gel electrophoresis of thylakoid membranes isolated from leaves of rye illuminated with low (150 μmol m^{-2} s^{-1}) or high (1200 μmol m^{-2} s^{-1}) light for 3 h. The spectra were normalized at their maximum. The excitation wavelength was at 470 nm. Panels B and D: difference spectrum; spectrum of the LHCII complexes isolated from the high light illuminated plants minus spectrum of the LHCII complexes isolated from the low light illuminated plants. The width of the slits was 5 nm.