Biochimica et Biophysica Acta 1777 (2008) 425–432

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants

Mikko Tikkanen [a], Markus Nurmi [a], Marjaana Suorsa [a], Ravi Danielsson [b], Fikret Mamedov [c], Stenbjörn Styring [c], Eva-Mari Aro [a,*]

[a] Plant Physiology and Molecular Biology, Department of Biology, University of Turku, FIN-20014 Turku, Finland
[b] Department of Biochemistry, Center for Chemistry and Chemical Engineering, P.O. Box 124, Lund University, 221 00 Lund, Sweden
[c] Molecular Biomimetics, Department of Photochemistry and Molecular Science, Ångström Laboratory, Box 523, Uppsala University, Uppsala 75120, Sweden

ARTICLE INFO

Article history:
Received 7 December 2007
Received in revised form 7 February 2008
Accepted 8 February 2008
Available online 19 February 2008

Keywords:
LHCII
Photosystem I
Photosystem II
Photosynthesis
Thylakoid protein phosphorylation
State transition

ABSTRACT

Phosphorylation-dependent movement of the light-harvesting complex II (LHCII) between photosystem II (PSII) and photosystem I (PSI) takes place in order to balance the function of the two photosystems. Traditionally, the phosphorylatable fraction of LHCII has been considered as the functional unit of this dynamic regulation. Here, a mechanical fractionation of the thylakoid membrane of *Spinacia oleracea* was performed from leaves both in the phosphorylated state (low light, LL) and in the dephosphorylated state (dark, D) in order to compare the phosphorylation-dependent protein movements with the excitation changes occurring in the two photosystems upon LHCII phosphorylation. Despite the fact that several LHCII proteins migrate to stroma lamellae when LHCII is phosphorylated, no increase occurs in the 77 K fluorescence emitted from PSI in this membrane fraction. On the contrary, such an increase in fluorescence occurs in the grana margin fraction, and the functionally important mobile unit is the PSI–LHCI complex. A new model for LHCII phosphorylation driven regulation of relative PSII/PSI excitation thus emphasises an increase in PSI absorption cross-section occurring in grana margins upon LHCII phosphorylation and resulting from the movement of PSI–LHCI complexes from stroma lamellae and subsequent co-operation with the P-LHCII antenna from the grana. The grana margins probably give a flexibility for regulation of linear and cyclic electron flow in plant chloroplasts.

1. Introduction

Photosynthetic pigment protein complexes are embedded in the thylakoid membrane of plant chloroplasts. These membranes are structurally complex, highly organized and dynamically regulated structures [1–3]. Stacked grana membranes are connected via non-appressed margins to the stroma membranes. Shade and sun plants differ in their chloroplast ultrastructure. Shade plants have evolved extensive granal stacking as an adaptation to low light conditions and, conversely, the sun plants have less appressed thylakoids [4]. Moreover, the thylakoid membrane organization can rapidly and reversibly respond to changes in light intensity and quality, and importantly, this regulation follows similar light dependent dynamics as the phosphorylation of LHCII [5]. In addition to this complex organization and behaviour, the two photosystems are laterally segregated in the thylakoid membrane: PSI–LHCI is located in non-appressed stroma regions whereas PSII–LHCII is predominantly located in the stacked grana core [6,7].

The PSII supercomplex is composed of a dimeric core complex of PSII, which is associated with two copies of each minor light-harvesting proteins, two strongly bound LHCII trimers and two or more less tightly bound trimers [8–10]. PSI is a monomeric complex in higher plants composed of 14 core proteins and 5 Lhca antenna proteins [11]. Light-harvesting antenna complexes in the thylakoid membrane absorb sunlight and transfer the excitation energy to PSII and PSI core complexes in order to drive electron transport. The light-harvesting antenna of PSII consists of at least six different chl-binding proteins Lhcb1-6 [12] and can serve both the PSII and PSI reaction centers via state transition [13; reviewed in Ref. 14]. LHCII, the major antenna complex of PSII, is formed of trimers [15] of three proteins; Lhcb1, Lhcb2 and Lhcb3 in different combinations.

On the stromal side of the thylakoid membrane, the N-terminal threonine residue of the Lhcb1 and Lhcb2 proteins is prone to reversible phosphorylation [16] regulated by the redox conditions in the thylakoid membrane and the surrounding stroma [17, reviewed in Ref. 18]. Lhcb phosphorylation is needed for reversible translocation of a fraction of LHCII between PSII and PSI. The reaction is catalysed by the STN7 kinase [19–22]. Three minor chl-binding proteins, Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24), are monomeric, and among these Lhcb4 is the only phosphoprotein in plants [23]. Phosphorylatable Lhcb1 and Lhcb2 are the most abundant antenna proteins and they are considered to form the antenna fraction (P-LHCII) moving between PSI and PSII. Dephosphorylated LHCII serves only PSII and when phosphorylated it is, at least partially, connected to the PSI core

* Corresponding author. Tel.: +358 2 333 5931; fax: +358 2 333 8075.
E-mail address: evaaro@utu.fi (E.-M. Aro).

0005-2728/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2008.02.001

complex via the PsaH, PsaL and PsaO subunits, and indeed these minor PSI proteins are a prerequisite for the mobility of P-LHCII [24]. Studies on PSI mutants lacking one of the essential subunits for P-LHCII association with PSI have revealed a more complex nature of P-LHCII movement between the two reaction centers than was previously envisaged and which was simply based on the phosphorylation-induced movement of LHCII to the stroma thylakoids [25].

In this study, using a non-invasive mechanical membrane fractionation and two-phase partition method, we isolated five different fractions of the thylakoid membrane: the grana core, the grana margins, entire grana containing both the grana core and grana margins, the stroma lamellae, and deep stroma lamellae fraction called Y-100 [7,26]. These fractions were isolated both from thylakoids of dark acclimated plants (D) where LHCII is dephosphorylated and from plants after 2 h of low light acclimation (LL), earlier shown to induce maximal LHCII phosphorylation [27]. Obtained results prompted us to present a new model for LHCII phosphorylation-dependent regulation of excitation energy distribution between PSII and PSI.

2. Materials and methods

2.1. Growth of plants and fractionation of the thylakoid membrane

WT *Arabidopsis thaliana* (L.) ecotype Columbia (Col-0) and *stn7* mutant [22] plants were grown in phytotron under 120 μmol photons m^{-2} s^{-1}, 8 h photoperiod. Plants were exposed to light favoring the excitation of PSII (PSII light) and that of PSI (PSI light) to ensure the maximal phosphorylation and dephosphorylation of thylakoid proteins, respectively. A fluorescent tube (GroLux F58W/GROT8 Sylvania) covered with orange filter (Lee 105 filter, Lee Filters) served as PSII light and PSI light was obtained from halogen lamps (500 W) covered with an orange filter (Lee 105, Lee Filters) and a 'Median blue' filter (Roscolux # 83, Rosco Europe). Temperature was maintained at 23 °C by water-cooled glass chamber between the fluorescence tube and the plants.

Spinach plants (*Spinacia oleracea* L.) were grown hydroponically under cool white fluorescent lamps (Osram HQI-E400W/DV, Germany) at 20 °C with light/dark periods of 12 h and with the light intensity of 300 μmol photons m^{-2} s^{-1}. Before harvesting of the leaves, two month old plants were dark adapted for 24 h to fully dephosphorylate LHCII (D) or treated with 100 μmol photons m^{-2} s^{-1} for 2 h (LL), which induces the maximal phosphorylation of LHCII in spinach plants grown at 300 μmol photons m^{-2} s^{-1} [27]. Thylakoid fractionation was then performed according to Refs. [7,26]. All preparation procedures were made under weak green light at 4 °C, and the sample was kept on ice throughout the whole isolation process. All thylakoid fractions were prepared without any detergent to preserve the membrane as intact as possible. The membranes were mechanically broken by sonication and then submitted to an aqueous two-phase system to isolate the grana and stroma lamellae fractions as described in [7,28,29]. The grana fraction was further purified according to [7,30] to isolate the grana core fraction and the grana margin fraction, which does not contain end membranes. The Yeda press treatment of the thylakoid membrane followed by centrifugation steps according to [7,31,32] resulted in the Y-100 fraction, which is considered to be the most purified stroma lamellae fraction. The contents of chl a and b were determined according to Ref. [33].

2.2. SDS-PAGE, phosphoprotein staining and immunoblotting

Proteins of the thylakoid membrane and different membrane subfractions were separated by SDS-PAGE using 15% (w/v) acrylamide gels with 6 M urea [34]. Pro-Q Diamond Phosphoprotein Gel Stain and SYPRO® Ruby Protein Gel Stain (Molecular Probes) were used according to manufacture's instructions after determining the sample concentrations that give a linear response after staining. Membranes equivalent to 0.05 μg of chl a were loaded in gels to investigate the most abundant proteins and 0.8 μg of chl a was loaded to investigate the less abundant thylakoid proteins.

For immunoblotting, the amount of proteins loaded in the gels was tested for each antibody to give a linear immunoresponse. Accordingly, the protein amounts corresponding from 0.2 to 3.2 μg of chl were loaded in the gels, depending on the antibody used. The polypeptides were transferred to an Immobilon-P membrane (Millipore, Bedford, MA), and the membrane was blocked with 5% (w/v) milk (Bio Rad) or with 5% fatty-acid free bovine serum albumin (Sigma-Aldrich) for P-thr antibody (New England Biolabs). Western blotting was performed with standard techniques using protein-specific antibodies purchased from Agrisera (Vännas, Sweden) (Lhcb1, Lhcb2, Lhcb3, CP26 (Lhcb5), CP24 (Lhcb6), PsaH, PsaL Lhca1, Lhca2, Lhca3, Lhca4, PsbS) and other antibodies as described previously [35,36]. Proteins were immunodetected using a Phototope-Star Chemiluminescent kit (New England Biolabs).

2.3. 77 K fluorescence emission and fluorescence excitation spectra

Thylakoid membranes and different thylakoid subfractions were diluted in buffer containing 50 mM Hepes/KOH, pH 7.5, 100 mM sorbitol, 10 mM $MgCl_2$, and 10 mM NaF to a chl concentration of 10 μg/ml. Then samples were immediately frozen and 77 K fluorescence emission spectra were recorded with a diode array spectrophotometer (S2000; Ocean Optics, Dunedin, FL, USA) equipped with a reflectance probe. To record the 77 K fluorescence emission curves the samples were excited with white light below 500 nm, defined by LS500S and LS700S filters (Corion Corp., Holliston, MA, USA). To obtain the 77 K fluorescence excitation spectra, the samples were excited with wavelengths from 400 to 540 nm with 5 nm steps by using f/3.4 Monochromator (Applied Photophysics, Surrey, U.K.). The emission between 600 and 800 nm was recorded (see Figs. 1 and 3 legends for details).

3. Results

3.1. Relation between LHCII phosphorylation and the relative excitation of PSI and PSII

Generally, different approaches have been taken to dephosphorylation or phosphorylation of the LHCII depending on the organism under study. In *Chlamydomonas*, light independent methods have been used most often [37], whereas in spinach plants the dark and low light treatments efficiently induce full dephosphorylation and phosphorylation of LHCII proteins, respectively [27]. On the contrary, for *Arabidopsis* the use of light qualities that selectively excite either PSI or PSII is the best choice. As shown in Fig. 1, the relative distribution of excitation energy between PSII and PSI is dependent on the phosphorylation of LHCII proteins. When LHCII is phosphorylated, the 77 K PSI/PSII (F733 nm/F685 nm) fluorescence ratio is higher compared to the dephosphorylated state (Fig. 1A). On the contrary, the *stn7* mutant, which cannot phosphorylate LHCII, keeps the relative excitation of PSI and PSII nearly constant during the whole 120 min treatment with the PSI and PSII lights (Fig. 1A). In fact, the *stn7* mutant is inert for even longer treatments with PSI, PSII or white light [22]. Under normal growth conditions the *stn7* mutant synthesizes more PSI–LHCI proteins compared to WT [22] in order to compensate the lack of energy donation from P-LHCII to PSI. This increases the 77 K F733/F685 ratio

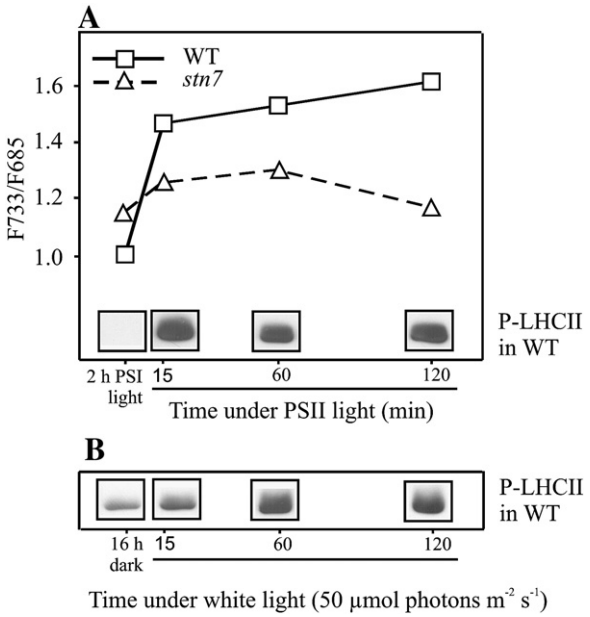

Fig. 1. Relationship between LHCII phosphorylation and 77 K fluorescence emission ratio (733 nm /685 nm) in WT and the *stn7* LHCII kinase mutant of *Arabidopsis*. A. WT and *stn7* plants were first treated for 120 min with light favoring PSI excitation (PSI light) and then transferred under light favoring PSII excitation (PSII light) for 15, 60 and 120 min. Phosphorylation level of LHCII proteins was detected by P-thr antibody (WT shown, no phosphorylation detected from *stn7*) and the amplitudes of 77 K fluorescence peaks (733 nm and 685 nm) were measured. All the 77 K samples were prepared in the presence of 10 mM NaF and diluted to 10 μg of chl/ml before the measurements. B. Kinetics of LHCII phosphorylation, as detected by P-thr antibody, in WT *Arabidopsis* upon illumination by low intensity of white light. Thylakoids were isolated after 16 dark treatment and after 15, 60 and 120 min illumination with white low light of 50 μmol photons m^{-2} s^{-1}. Thylakoids were isolated in the presence of 10 mM NaF.