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Fe deficiency induces phosphorylation and translocation of Lhcb1 in barley thylakoid membranes

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ABSTRACT

HvLhcb1 a major light-harvesting chlorophyll *a*/*b*-binding protein in barley, is a critical player in sustainable growth under Fe deficiency. Here, we demonstrate that Fe deficiency induces phosphorylation of HvLhcb1 proteins leading to their migration from grana stacks to stroma thylakoid membranes. HvLhcb1 remained phosphorylated even in the dark and apparently independently of state transition, which represents a mechanism for short-term acclimation. Our data suggest that the constitutive phosphorylation-triggered translocation of HvLhcb1 under Fe deficiency contributes to optimization of the excitation balance between photosystem II and photosystem I, the latter of which is a main target of Fe deficiency.

1. Introduction

There are many heme- and Fe–S cluster-containing proteins working in photosynthetic electron transport chain so that iron (Fe) deficiency tends to cause severe photodamage [1]. Photosystem I (PSI) core components contain three [4Fe–4S] clusters, which bind quite a large proportion of Fe present in thylakoid membranes. Thus, the relative amount of PSI complexes decreases under Fe deficiency with smaller effect on Photosystem II (PSII) [2]. Once the function of PSI deteriorates as a result of Fe deficiency, photoinhibition of PSII could be induced even under moderate photosynthetic photon flux density (PPFD) by the over-reduction of electron transfer components between PSI and PSII [3]. To avoid the damage due to the decrease of PSI reaction center complexes, cyanobacteria and eukaryotic algae induce light-harvesting antenna complexes surrounding PSI under Fe-deficiency in order to compensate for the reduced levels of PSI activity or to protect photosystems from excess light [4–6]. In contrast to these organisms, there is no evidence that terrestrial plants induce such specific PSI antenna proteins to optimize the distribution of photosynthetic excitation energy between the two photosystems under Fe deficiency.

We have recently found that Fe-deficiency specifically induces a gene of the LHCII family that encodes a homolog of Lhcb1 in barley (*Hordeum vulgare*), a plant that is tolerant to Fe deficiency [7,8]. Although the HvLhcb1 protein is the most abundant light-harvesting antenna protein of PSII, the main role of HvLhcb1 in Fe-deficient barley is to prevent the capture of excess energy, and thus decrease the electron flow from PSII to PSI via thermal dissipation (non-photochemical quenching) under Fe-deficient conditions [7]. However, the mechanism by which HvLhcb1 decreases electron transfer from PSII to PSI has not been determined. In this study, we determined the distribution and phosphorylation status of HvLhcb1 protein in Fe-deficient barley. The results demonstrate that a large proportion of HvLhcb1 is located adjacent to PSI rather

Abbreviations: Lhcb1, a light-harvesting chlorophyll *a*/*b*-binding protein of photosystem II; LHC, light-harvesting complex; LHCII, light-harvesting complex of photosystem II; PSI, photosystem I; PSII, photosystem II; NPQ, non-photochemical quenching; Chl, chlorophyll; PPFD, photosynthetic photon flux density; TEM, transmission electron microscopy; PAM, pulse amplitude modulation; *Fv*/*Fm*, maximum quantum yield of photosystem II; *qT*, a quench component related to state transition; FR, far-red

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than PSII under Fe-deficient conditions. Apparently, phosphorylation of HvLhcb1 is responsible for the translocation. The series of events would result in a decreased rate of electron transport from PSII to PSI, which in turn appears to contribute to optimize the excitation balance between PSII and PSI, the latter of which decreases as a main target of Fe deficiency.

2. Materials and methods

2.1. Plant materials

Seeds of the barley plant (*Hordeum vulgare* L. cv. 'Ehimehadaka No. 1') were germinated in moist filter paper, and the seedlings were grown hydroponically as previously described [7]. Plants were grown at 24 ± 2 °C and a PPFD of 200 μmol m^{-2} s^{-1} under a 14 h light/10 h dark cycle. Ten-day-old seedlings were transferred to newly prepared hydroponic solution containing 0 or 30 μM ethylenediamine-N,N,N′,N′-tetraacetic acid–Fe. We used the 5th to 7th young developed leaves of the barley plant which was grown with nutrient solution with or without Fe for 20–28 d.

2.2. Transmission electron microscopy (TEM)

Small pieces of leaves were fixed with 4% glutaraldehyde and 4% formaldehyde in sodium phosphate buffer (0.1 M, pH 7.4) under deaerated conditions, and were washed three times with the same buffer containing 10% (w/v) sucrose. Samples were then post-fixed with 2% OsO_4. After dehydration with a graded ethanol series and subsequently 100% propylene oxide, specimens were embedded in EPON 812 (Polysciences, PA, USA) and polymerized. Ultrathin sections (70 nm) were cut using an Ultracut UCT microtome (Leica, Germany), and these were stained with 2% uranyl acetate and lead stain solution (Sigma Aldrich Co., MO, USA). TEM was performed using a JEM-1400 plus transmission electron microscope (JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 80 kV. Images were captured using a CCD camera (VELETA; Olympus Soft Imaging Solutions GmbH).

2.3. Thylakoid fractionation

Thylakoid membranes were isolated after the isolation of intact chloroplasts according to our previous study [7]. NaF (10 mM) was added to the thylakoid washing buffer for the analysis of LHCII phosphorylation. The grana and stroma membranes were fractionated using a modified dual-detergent method [9]. The grana core membranes, margins, and stroma lamellae were re-suspended in a washing buffer, flash frozen in liquid nitrogen, and stored at −80 °C until use.

2.4. Fluorescence emission spectra at 77 K

Low-temperature fluorescence emission spectra at 77 K were recorded using a custom-made apparatus [10]. Isolated thylakoid membranes [5 μg chlorophyll (Chl) per mL] were frozen in the dark with liquid nitrogen. Pigments were excited with blue light passing through a band-pass filter (Corning CS 4-96) and a dichroic filter (DF Blue; Optical Coatings Japan, Tokyo, Japan).

2.5. SDS–PAGE, immunoblotting, and analysis of phospho-proteins

Proteins of thylakoid membranes were solubilized in protein-solubilizing buffer and subjected to SDS–PAGE [11]. An immunoblot analysis was performed as previously described [7]. The antibodies were obtained from Agrisera (Vännäs, Sweden) and Prof. I. Enami (Tokyo University of Science). To detect phosphorylation of the thylakoid membrane proteins, Phos-tag® acrylamide (Wako, Tokyo, Japan) were used according to the manufacturer's protocol.

2.6. Pulse amplitude modulation (PAM) Chl fluorescence measurements

Chl fluorescence parameters (*Fv*/*Fm*, NPQ and ETR) were determined by PAM fluorimeter using an integrated fluorescence chamber head (LI-6400-40, LI-COR Inc., NE, USA) attached to LI-6400XT as previously described [7].

2.7. Analysis of state transition

The level of state transitions were determined with a LI6400-XT (LI-COR) portable system equipped with a 6400-40 sensor head, according to Ruban and Johnson [12]. Undetached leaves from 30-min dark-adapted plants were used for measurements. The maximum fluorescence yield (*Fm*) was determined by applying a saturating flash (900 ms, 12,000 μmol m^{-1} s^{-1}). The leaves were illuminated for 15 min by far-red (FR) light LED of a 6400-40 leaf chamber head (740 nm) for PSI excitation. The maximum fluorescence yield under State I (*Fm1*) was determined at the end of this illumination. Subsequently, State II was induced by PSII light (20 μmol m^{-1} s^{-1}) from LEDs (635 nm) for 15 min. At the end of this illumination, the maximum fluorescence yield under State II (*Fm2*) was determined. Levels of transition from State I to State II were quantified as follows: $qT\ (\%) = (Fm1-Fm2)/Fm1 \times 100$.

3. Results

3.1. Most of the thylakoid membranes from Fe-deficient barley leaves were unstacked

TEM observations of the chloroplasts of Fe-deficient barley showed that they had abundant thylakoid membranes and contained the normal number of starch granules, although the number and size of plastoglobules (lipid bodies) were significantly increased compared with Fe-sufficient barley (Fig. 1). The observed structural changes in barley chloroplasts are different from those in the chloroplasts of other Fe-deficient plant species, such as maize [13] and sugar beet [14], or in several mutants of *Arabidopsis thaliana* showing Fe-deficiency symptoms [15,16]. In these plant species, in contrast to Fe-deficient barley, the development and function of chloroplasts are distorted under Fe deficiency, characterized by inchoate or absent thylakoid membranes and a significant decrease in the number of starch granules [13–16]. These results suggest that barley, a plant that is highly tolerant to Fe deficiency, has ability to maintain CO_2 assimilation under Fe-deficient conditions, as was found in our previous work [7]. Judging from the normal amounts of membranes and the increased number of plastoglobules, lipid synthesis in chloroplasts also seems to be functional during Fe deficiency.

On the other hand, granum formation in chloroplasts was strongly perturbed by Fe deficiency. Since LHCII is responsible for membrane stacking in thylakoid membranes [17,18], we initially assumed that the granum formation in barley thylakoid membranes would be accelerated under Fe-deficient conditions, in which barley should contain a greater number of HvLhcb1 proteins on the Chl basis [7]. Furthermore, PSI-enriched stroma membrane would decrease in Fe-deficient leaves, since the amount of PSI complexes decreases under Fe deficiency [7]. Contrary to our expectations, however, observations of chloroplast ultrastructure revealed that a large proportion of the thylakoid membranes was unstacked or loosely interacting in Fe-deficient barley leaves (Fig. 1B, D, and F) compared with those in Fe-sufficient leaves

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