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SHORT COMMUNICATION

Requirement of ONE-HELIX PROTEIN 1 (OHP1) in early *Arabidopsis* seedling development and under high light intensity

Daniel Hey and Bernhard Grimm

Lebenswissenschaftliche Fakultät, Institut für Biologie, AG Pflanzenphysiologie, Humboldt-Universität zu Berlin, Berlin, Germany

ABSTRACT

Plant ONE-HELIX PROTEINS (OHPs) are part of the light-harvesting complex superfamily whose members are involved in various processes related to sensing and capturing light as well as light protection. We recently showed the requirement of a functional OHP1-OHP2 heterodimer for efficient D1 synthesis. Interestingly, while the *ohp1* knockout mutant showed a strong defect in accumulation of the photosystem II and is hardly viable, virus-induced gene silencing of *OHP1* had no detectable impact on plant growth and performance under standard growth conditions. However, *in vivo* labeling assays with ^{35}S-methionine indicate a reduced D1 synthesis rate. Here, we show that VIGS-OHP1 plants are more susceptible towards elevated light intensities than control plants. This underlines an obligatory function of OHP1 for light acclimation.

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Emerging insights into the function of the *Arabidopsis* one-helix proteins (OHPs) emphasize the important impact of OHPs on the accumulation of the photosystem II (PSII) core complex proteins[1-3] rather than a functional association of OHP2 with PSI, which was initially proposed.[4] However, a high light (HL)-induced increase in *OHP1* transcripts was reported[2,5] and OHP1 was postulated to be functionally correlated with the response to light stress. As it is currently assumed that both OHP variants act as OHP1-OHP2 heterodimers, their function depends on the mutual stability of both proteins.[1] Virus-induced gene silencing (VIGS) of *OHP2* phenotypically and biochemically resembled the *ohp2* T-DNA insertion mutant lines. They were characterized by an almost undetectably slow D1 synthesis rate, leading to a specific destabilization of PSII core proteins and PSII complexes.[1] In contrast, VIGS-OHP1 plants showed no phenotypical modification relative to the VIGS-GFP (green fluorescent protein) control seedlings under standard growth conditions as well as control-like levels of PSII proteins, although also in these plants the D1 synthesis rate was decreased.[1]

We continued to explore the impact of OHP1 on the D1 synthesis rate and stability and exposed the VIGS-plants to light stress conditions: Leaf discs of VIGS-plants were excised and exposed to HL (850 µmol photons s^{-1} m^{-2}) while floating on ultrapure water and being protected from the heat of the fluorescent lamps by a 5 cm water layer in a transparent container to prevent withering of the samples. Samples were harvested at the beginning as well as after 2, 6 and 24 hours of HL exposure and chlorophyll fluorescence was measured with a PAM imager after a 10 minute dark incubation (Figure 1(a)). Both VIGS-GFP and VIGS-OHP1 showed a similar Fv/Fm ratio at the beginning of the experiment, but in VIGS-OHP1 the Fv/Fm ratio decreased much faster upon HL exposure than in the VIGS-GFP control (Figure 1a, b)). After 24 hours of HL exposure the Fv/Fm ratio in both samples partially recovered, but still retained lower in VIGS-OHP1. To assess D1 levels, total proteins were separated by SDS-PAGE and analyzed by immunoblotting (Figure 1(c)). Whereas VIGS-GFP seedlings contained a stable amount of D1 during 24 hours of HL, D1 remarkably decreased after 6 hours of treatment in VIGS-OHP1 seedlings and remained at a low level until the end of the experiment. As reported earlier,[1] the OHP2 content decreased in VIGS-OHP1 leaf samples, while the content of OHP2 and OHP1 slightly increased in control seedlings during HL stress (Figure 1(c)).

The increased OHP1 content during the short-term HL exposure is in line with previous reports indicating a HL-dependent induction of *OHP1* expression.[2,5] When re-monitoring the expression of *OHP1* and *OHP2* by RT-qPCR, the VIGS-GFP leaf discs showed twice the amount of both transcripts after 24 hours of HL (Figure 2). We compared this result with other known HL-inducible genes, here for example *ELIP1*, which encodes EARLY LIGHT-INDUCIBLE PROTEIN 1, another member of the LHC protein family. However, the *ELIP1* transcripts accumulated more than 100-fold after 24 hours of HL exposure (Figure 2). In comparison, the twofold increased levels of the *OHP1* transcript seemed to be marginal. Thus, it remains open whether the observed increased OHP content (Figure 1(c)) corresponds to increasing transcript levels or is caused by HL-triggered accumulation or stabilization of OHPs. A decrease of the Fv/Fm ratio upon HL exposure, which indicates a lower maximum quantum efficiency of PSII, was determined for both lines, VIGS-GFP and VIGS-OHP1 (Figure 1(a, b)). Generally, a decreasing Fv/Fm ratio counts for stress exposure, such as HL, and is caused by damage or

CONTACT Bernhard Grimm bernhard.grimm@rz.hu-berlin.de Lebenswissenschaftliche Fakultät, Institut für Biologie, AG Pflanzenphysiologie, Humboldt-Universität zu Berlin, Philippstrasse 13, Berlin 10115, Germany

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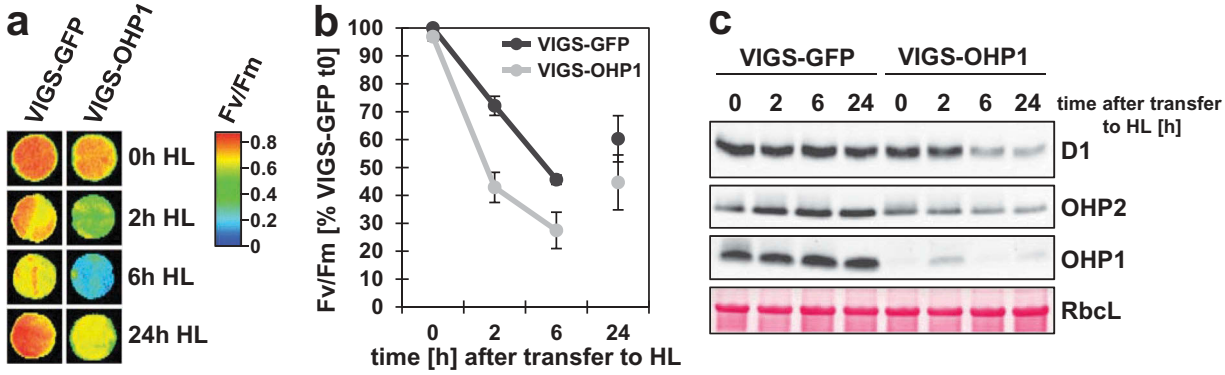

Figure 1. VIGS-OHP1 plants are susceptible to high light intensities.

(a) Measurement of Fv/Fm ratios in leaf discs of VIGS-GFP and VIGS-OHP1 plants after exposure to high light (850 µmol photons s^{-1} m^{-2}). False colors indicate Fv/Fm ratios and numerical values of the color code are indicated. One representative leaf disc for each time-point is shown. (b) Numeric representation of Fv/Fm ratios displayed in A. Data are normalized to Fv/Fm in VIGS-GFP at time-point t_0, which is set to 100%. Mean values and standard deviations of three biological replicates are shown. B, Western blot analysis of OHP variants and D1 from leaf discs of VIGS-GFP and VIGS-OHP1 plants exposed to HL as described above. Ponceau S staining of the large subunit of RuBisCO (RbcL) is shown as the loading control.

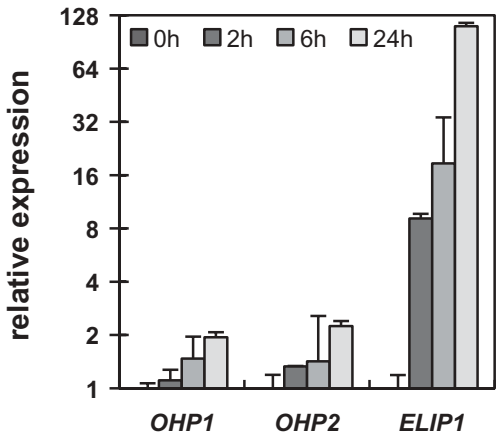

Figure 2. Light-induced expression of *OHP* transcripts.

RT-qPCR analysis of *OHP1*, *OHP2* and *ELIP1* transcripts in VIGS-GFP plants during exposure to HL. Data were analyzed by the ΔΔCt-method and expression was normalized to the first time-point (t_0). A mean value of three biological replicates is shown, error bars represent standard deviation.

inactivation of PSII, *i.e.* photoinhibition, or induced non-photochemical quenching.[6] The faster decreasing Fv/Fm ratios of VIGS-OHP1 compared to those of VIGS-GFP seedlings indicate a higher susceptibility of PSII towards excessive amounts of light in seedlings with OHP deficiency. *In-vivo* labeling assays with ^{35}S-methionine unveiled a lower synthesis rate of D1 in VIGS-OHP1,[1] which is consequently also consistent with a strong decline of the D1 content after the HL periods (Figure 1(c)). In conclusion, impaired D1 recycling and/or lower stability correlate with the deficiency of OHP1 under HL conditions.

Besides the eukaryotic OHPs, the other members of the LHC superfamily possess one or two LHC-transmembrane helices. The cross-like configuration of these helices in the LHCPs and presumably also ELIPs contributes to the stability of the proteins.[7] By analogy, the two OHP variants are proposed to interact with each other to allow binding of chlorophyll and/or precursors between the two transmembrane domains. It is proposed that the heterodimer formation is crucial for stability and function of both OHPs. Derived from the *ohp1* and the HL-exposed VIGS-OHP1 phenotype, OHP1 is predicted to be indispensable for effective functioning of the OHP1-OHP2 heterodimer during early seedling development and in adult plants under light stress. The early developmental stage is characterized by a rapid assembly of the photosynthetic complexes in a vulnerable environment, which resembles a light-sensitive state. Light stress conditions in adult plants require a high rate of the D1 repair cycle.[8] The complete set of (pigment supplying) auxiliary factors, including OHPs and HCF244[9] might be essential for acclimation of plants at both conditions. Thus, the seedling-lethal phenotype of the *ohp1* mutant and the stronger susceptibility of photosynthesis to HL-stress in the VIGS-OHP1 lines are explained with impaired action of the OHP heterodimer. Ultimately, the putative pigment-binding ability of the OHP-complex favors the hypothesis that the OHP1-OHP2 heterodimer formation is required to deliver chlorophyll for newly synthesized D1 proteins and to quench excitation energy of chlorophyll during a salvage pathway.[10–13]

Materials and methods

Plant material and growth conditions, vigs-assay

Arabidopsis wild-type plants (ecotype Col-0) were grown on soil in a growth chamber under long day conditions (16h photoperiod, 100µmol photons s^{-1} m^{-2}, 20°C). The VIGS-assay was performed with 12-day-old seedlings as previously described.[1]

Light-stress assay

Leaf discs (7mm diameter) were excised from 5-week-old VIGS plants and incubated in HL (850 µmol photons s^{-1} m^{-2}) for up to 24 hours floating on ultrapure water. To shield the discs from heat radiation of the lamps, a 5cm thick water basin was

placed on top of the samples. Samples were harvested at indicated time-points.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence kinetics of leaf discs were measured in a PAM-imager chamber (FluorCam 700MF, Photon Systems Instruments) after ten minutes of dark incubation. Fv/Fm ratios were normalized to time-point T_0 of VIGS-GFP and displayed in per cent.

SDS-PAGE, Western blot

Total leaf protein extraction, SDS-PAGE, Western blotting and chemiluminescence detection was performed exactly as previously described.[1]

RNA extraction, RT-qPCR

Total RNAs were extracted by a citric acid based extraction method[14] and cDNA-synthesis, reverse transcription and RT-qPCR was performed exactly as described before.[1] Data were analyzed by the ΔΔCt-method.[15]

Abbreviations

GFP	green fluorescent protein
HL	high light
OHP	one-helix protein
PAM	pulse-amplitude modulation
PS	photosystem
RbcL	large subunit of Ribulose-1,5-bisphosphate carboxylase/oxygenase
VIGS	virus-induced gene silencing

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ORCID

Daniel Hey http://orcid.org/0000-0002-8749-8352
Bernhard Grimm http://orcid.org/0000-0002-9730-1074

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