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A novel light stress-enhanced one-helix chlorophyll *a/b*-binding protein from *Arabidopsis thaliana*

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Introduction

The family of chlorophyll *a/b*-binding (Cab) proteins in higher plants is composed of over 20 different members associated with photosystem I (PSI) or photosystem II (PSII). All known Cab proteins are nuclear-encoded and posttranslationally inserted into thylakoid membranes. Based on the three-dimensional structure determined at 3.4 Å resolution for one member of the Cab family (Kühlbrandt et al., 1994) it was proposed that all light-harvesting chlorophyll *a/b*-binding proteins (Lhcp) in higher plants and green alga have three transmembrane α-helices, where helices I and III are held together by ion pairs formed by charged residues.

In the past years, several potential chlorophyll-binding proteins with transient expression pattern have been described from higher plants, algae or cyanobacteria (reviewed in Adamska, 1997, 2001). These distant relatives of Cab proteins were included into a subfamily of proteins called Elip (early light-induced proteins). The members of Elip subfamily can be divided into three groups depending on the number of transmembrane helices and pattern of gene expression under light stress conditions. In *Arabidopsis thaliana* three-helix Elips, two-helix Seps (stress-enhanced proteins) and one-helix Ohps (one-helix proteins) have been described (reviewed in Adamska, 2001). While Elips were shown to be absent in thylakoid membranes isolated from plants grown under ambient light conditions (Adamska et al., 1992; Heddad and Adamska, 2000), the Seps and Ohps were detected in the absence of light stress but their level increased significantly after exposure of leaves to high irradiance (Heddad and Adamska, 2000; Andersson and Adamska, this proceedings). No definite function has yet been reported for Elips. It was proposed that the members of Elips family might play a protective role within the thylakoids under light stress conditions either by transient binding of free chlorophyll molecules and preventing the formation of free radicals and/or by acting as sinks for excitation energy (reviewed in Montané and Kloppstech, 2000; Adamska, 2001).

In this work we isolated a novel member of Elip family in *Arabidopsis* with one predicted transmembrane α-helix, which we called Ohp2 (for a second one-helix Cab protein described from higher plants). We demonstrated that the *Ohp2* gene is expressed under low light conditions but the amounts of Ohp2 transcript and protein increased significantly after exposure of leaves to light stress.

Materials and methods

Growth of plants and light stress conditions.

Arabidopsis thaliana L. cv. Columbia plants were grown in a growth chamber on soil at 25°C at a light intensity of 100 $\mu mol \cdot m^{-2}s^{-1}$ under short-day conditions. Light stress treatment was performed on mature leaves, detached from 4-5 weeks-old plants, floated on water and exposed to a light intensity of 2.500 $\mu mol \cdot m^{-2}s^{-1}$ for 2 hours. Plant material was collected and either immediately used for extractions or frozen in liquid nitrogen and stored at –70°C for further preparations.

Gene cloning, sequencing and data analysis.

The *Ohp2* gene from *Arabidopsis* was amplified by polymerase chain reaction (PCR) using cDNA library obtained from Arabidopsis Biological Resource Center at Ohio State University. Specific PCR primers were designed on the basis of sequence present in the expressed sequence tag (EST) cDNA database (TC87219). The amplified 519 bp (including stop codon) fragment corresponding to the coding region of Ohp2 cDNA was cloned into a pCR2.1 vector using TA cloning kit (Invitrogen) and cloned insert verified by sequencing of both cDNA strands (CyberGene, Sweden). Similarity searches were done using the Advanced Blast program. Alignment of amino acid sequences was performed using ClustalW with manual correction of gaps. The transmembrane regions were predicted using the Dense Alignment Surface method (DAS). For prediction of a transit peptide the ChloroP program was used. All prediction programs can be found under http://ca.expasy.org/tools/

RNA and protein analysis. Total RNA was extracted from control or light stress treated leaves using a RNeasy mini kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's protocol. After separation of 5 µg RNA in 1.2% agarose gel, RNA was transferred to Hybond-N^+ membrane prior to the hybridization as described (Heddad and Adamska, 2000). Intact chloroplasts were isolated and separated into soluble stroma and thylakoid membrane fraction as described (Heddad and Adamska, 2000). For separation of peripheral and integral membrane proteins, the thylakoid membranes were incubated with 4 M urea for 30 min at 4°C and pH 11.0 in darkness under gentle stirring. After centrifugation for 15 min at 14.000 rpm the thylakoid pellet (containing integral membrane proteins) and the supernatant (containing peripheral membrane proteins) were separated by SDS-PAGE and Ohp2 content was analyzed by immunoblotting.

Results and discussion

A Blast search in the *Arabidopsis* EST database with Elip consensus sequence (Adamska, 2001) resulted in the identification of the gene encoding a novel Elip-like protein, called Ohp2. We designed primers based on the EST sequence and used PCR to amplify the coding region of the *Ohp2* gene from an *Arabidopsis* cDNA library. The amplified 519 bp cDNA sequence encoded a protein predicted to be composed of 172 amino acids. The Ohp2 protein contained an N-terminal transit peptide (amino acids 1-42) typical for proteins imported into chloroplasts and a strongly conserved domain present in all Cab proteins and their relatives. This domain is located in transmembrane α-helices I and III of Lhcps and Elips (for review see Adamska, 2001) and in the helix I of Seps (Heddad and Adamska, 2000) or Ohps (called also Hlips for high light-induced proteins or Scps, for small Cab proteins) described from Pro- and Eukaryota (Miroshnichenko-Dolganov et al., 1995; Funk and Vermaas, 1999; Adamska, 2001). A comparison of helix I from Ohp2 with corresponding helices present in Lhcps, Elips and Seps is shown in Fig. 1.

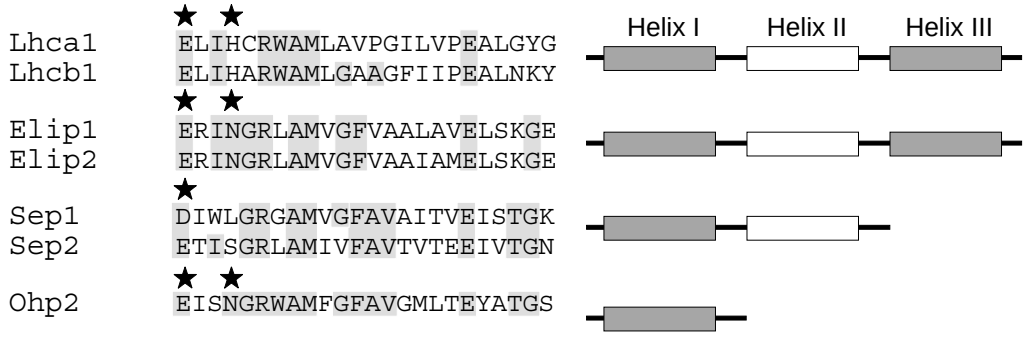

Figure 1. Predicted primary and secondary structure of Ohp2 from *Arabidopsis thaliana*. Sequence alignment of the conserved helix I of Lhca1 (GenBank accession: M85150), Lhcb1.1 (GenBank accession: X03907), Elip1 (GenBank accession: U89014), Elip2 (GenBank accession: Z97336), Sep1 (GenBank Accession: AF133716), Sep2 (GenBank accession: AF133717) and a novel Ohp2 protein (GenBank accession: AF378874) from *Arabidopsis.* The sequences were aligned manually with the assistance of the multiple alignment program CLUSTAL_W. Identical amino acids are shown on a grey background and conserved amino acid residues involved in chlorophyll ligation in LhcpII (Kühlbrandt et al., 1994) are marked by asterisks.

Hydropathy plots revealed that the mature Ohp2 is composed of 130 amino acids and differs from Elips and Seps by the presence of the only one transmembrane domain located at the C-terminus of this protein (Fig. 2A).

To confirm the chloroplast location of Ohp2 experimentally, we produced a polyclonal antibody against the recombinant protein and performed immunoblot analysis with purified intact chloroplasts or their subfractions (Fig. 2B). The results confirmed chloroplast localization of Ohp2. Further fractionation of chloroplasts into thylakoid membrane and stroma revealed that Ohp2 is located exclusively in the thylakoid membrane fraction (Fig. 2B). To prove an integral membrane location of Ohp2 isolated thylakoid membranes were washed with chaotropic agent urea to release extrinsic membrane proteins (Fig. 2C). The presence of Ohp2 in the thylakoid pellet confirmed an integral membrane location of Ohp2.

To analyze the gene expression of Ohp2, total RNA was isolated from leaves of *Arabidopsis* exposed to low light (control) or to high light conditions (light stress) and used for hybridization with Ohp2 specific cDNA probe. The results revealed (Fig. 2D, upper panel) that the transcript of Ohp2 was detected under low light conditions but its level increased during light stress tretament. This expression pattern of Ohp2 resembled those reported for *Sep1* and *Sep2* genes (Heddad and Adamska, 2000). In order to prove whether an increase in Ohp2 transcript level is accompanied by the accumulation of the corresponding protein immunoblot analysis were performed. The results showed (Fig. 2D, lower panel) that Ohp2 protein level in the thylakoid membranes was clearly enhanced by light stress treatment.

Thus, we conclude that in addition to the "classical" antenna proteins, the thylakoid membrane contains a large group (until now six different proteins of Elip family were reported from *Arabidopsis*) of potential pigment-binding proteins, which are induced in response to light stress. The function of these proteins remains to be elucidated.

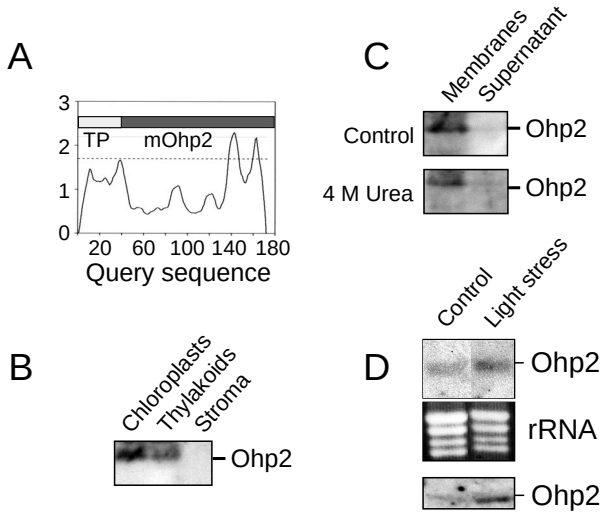

Figure 2. Localization and expression of Ohp2. (A) Hydropathy plot of the translated cDNA sequence of Ohp2. TP, transit peptide; mOhp2, mature Ohp2. (B) Thylakoid membrane location of Ohp2 assayed by immunoblotting. (C) Integral location of Ohp2 within the thylakoid membrane assayed by immunoblotting. (D) Expression of Ohp2 transcripts (upper panel) and protein (lower panel) under control and light stress conditions. As a reference, the rRNA pattern in the gel, visualized by staining with ethidium bromide, is shown.

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