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AtFtsH heterocomplex-mediated degradation of apoproteins of the major light harvesting complex of photosystem II (LHCII) in response to stresses

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ABSTRACT

Chloroplastic heterocomplex consisting of AtFtsH1, 2, 5 and 8 proteases, integrally bound to thylakoid membrane was shown to play a critical role in degradation of photodamaged PsbA molecules, inherent to photosystem II (PSII) repair cycle and in plastid development. As no one thylakoid bound apoproteins besides PsbA has been identified as target for the heterocomplex-mediated degradation we investigated the significance of this protease complex in degradation of apoproteins of the major light harvesting complex of photosystem II (LHCII) in response to various stressing conditions and in stress-related changes in overall composition of LHCII trimers of PSII-enriched membranes (BBY particles). To reach this goal a combination of approaches was applied based on immunoblotting, *in vitro* degradation and non-denaturing isoelectrofocusing. Exposure of *Arabidopsis thaliana* leaves to desiccation, cold and high irradiance led to a step-wise disappearance of Lhcb1 and Lhcb2, while Lhcb3 level remained unchanged, except for high irradiance which caused significant Lhcb3 decrease. Furthermore, it was demonstrated that stress-dependent disappearance of Lhcb1–3 is a proteolytic phenomenon for which a metalloprotease is responsible. No changes in Lhcb1–3 level were observed due to exposition of *var1-1* mutant leaves to the three stresses clearly pointing to the involvement of AtFtsH heterocomplex in the desiccation, cold and high irradiance-dependent degradation of Lhcb1 and Lhcb2 and in high irradiance-dependent degradation of Lhcb3. Non-denaturing isoelectrofocusing analyses revealed that AtFtsH heterocomplex-dependent differential Lhcb1–3 disappearance behaviour following desiccation stress was accompanied by modulations in abundances of individual LHCII trimers of BBY particles and that LHCII of *var1-1* resisted the modulations.

Introduction

Chloroplast proteases are thought to be intimately involved in numerous functions of photosynthetic apparatus by controlling quality and turnover of chloroplast proteins under changing environmental and ontogenetical conditions (for a review, see Wagner et al., 2012). In agreement with what may be expected based on the prokaryotic evolutionary origin of chloroplasts, their proteases are orthologs of bacterial ones. They involve proteolytical enzymes which are homologous to FtsH – the best characterized one among members of M41 family of *Escherichia coli* FtsH peptidases. FtsH from *E. coli* is an ATP-dependent, membrane-bound, 71-kDa metalloprotease posessing two transmembrane helices at its N-terminus, followed by an AAA^+ module and a proteolytical domain. The AAA^+ module bears motifs which are necessary for ATP binding and hydrolysis (Walker A, Walker B and an "arginine finger"), as well as substrate recognition, unfolding and translocation (FGV pore). The proteolytical domain contains zinc binding HEEXH motif (Narberhaus et al., 2009). Recent data suggest that bacterial FtsH is a hexameric assembly consisting of six 6-fold symmetric protease rings and six 2-fold symmetric AAA^+ rings (Bieniossek et al., 2009). FtsH degrades numerous cytoplasmic and membrane-bound protein substrates and is the only one among *E. coli* ATP-dependent proteases which is essential for the host survival under adverse environmental conditions (Bieniossek et al., 2006).

Twelve genes coding for proteins homologous to *E. coli* FtsH have been identified in *Arabidopsis thaliana* nuclear genome and labelled *AtFTSH1–12* (Sakamoto et al., 2003). The products of eight of them, namely AtFtsH1, 2, 5, 6, 7, 8, 9 and 12 have been shown to be targeted exclusively to chloroplasts (Sakamoto et al., 2003), AtFtsH11 seems to be dually targeted to chloroplast and mitochondria (Urantówka et al., 2005) whereas three other ones (AtFtsH3, 4 and 10) have been demonstrated to be targeted uniquely to mitochondria (Sakamoto et al., 2003). In spite of the fact that nine members of AtFtsH family may enter the chloroplasts only four of

Abbreviations: Chl, chlorophyll; LHCII, major light harvesting complex of photosystem II; ndIEF, non-denaturing isoelectrofocusing; PSII, photosystem II; SDS–PAGE, sodium–dodecyl sulphate gel electrophoresis.

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them – AtFtsH1, 2, 5 and 8 – have been demonstrated to accumulate at significant quantity, at thylakoid membrane (Sinvany-Villalobo et al., 2004). It is AtFtsH2 that under optimal conditions is the most abundant species followed by AtFtsH5, AtFtsH 8 and AtFtsH1, which accumulate to only 10% of FtsH2 (Sinvany-Villalobo et al., 2004). AtFtsH7, 9, 11 and 12 are significantly less abundant and localise to chloroplast envelope whereas localization of AtFtsH6 remains unknown (Ferro et al., 2010).

No crystal structure of chloroplast AtFtsHs has been resolved but data coming from various types of biochemical studies suggest that the pairs of the four most abundant subunits, namely AtFtsH1/5 (type A subunits) and AtFtsH2/8 (type B subunits) are members of the same multisubunit heterohexameric complex of thylakoid membrane, called AtFtsH heterocomplex (Sakamoto et al., 2003; Moldavski et al., 2012) although they use different thylakoid membrane integration pathways (Rodrigues et al., 2011). It was suggested that some pool of AtFtsH2 molecules may occur as homohexameric complexes as well (Sakamoto et al., 2003) but since double mutants lacking either both typeA or type both B subunits do not accumulate other FtsH subunits (Zaltsman et al., 2005b) it is believed that either type A or type B subunits may not assemble as homocomplexes or the supposedly formed homocomplexes are unstable (Moldavski et al., 2012). It has been suggested recently that chloroplast FtsH heterocomplex is present in spinach thylakoids as well, with hexameric form being by far the most prominent one in PSII-enriched grana membranes but with all possible forms from monomers to pentamers present in stroma thylakoids in low amounts as well. Furthermore, localization of spinach FtsH heterohexamers near the PSII–LHCII supercomplexes in the grana has been strongly suggested (Yoshioka et al., 2010).

AtftsH2 (*var2*) and *AtftsH5* (*var1*) mutants (both genetically identified by Martinez-Zapater, 1993) play a prominent role in studies concerning physiological functions of the AtFtsH heterocomplex. The most clearly demonstrated function of the AtFtsH heterocomplex is its involvement in degradation of photodamaged PsbA protein, inherent to PSII repair cycle. Namely, it was shown that mutants lacking AtFtsH2 and AtFtsH5 have lesser ability to deal with photoinhibition than wild type plants as judged by the results of Fv/Fm measurements (Sakamoto et al., 2002, 2003) and later it was demonstrated in a more direct way that both type A and type B subunits are required for selective PsbA turnover under noninhibitory and inhibitory conditions (Kato et al., 2009). Chloroplastic FtsH proteases not belonging to FtsH heterocomplex are implied to be involved in degradation of a few apoproteins other than photodamaged PsbA, *e.g.* AtFtsH6 has been suggested to be responsible for degradation of Lhcb1 apoprotein during high irradiance acclimation and of Lhcb3 during dark induced senescence (Żelisko et al., 2005), still, this AtFtsH6 function has been questioned recently (Wagner et al., 2011). An unidentified FtsH was suggested to be responsible for primary cleavage of spinach PsbA apoprotein under moderate heat stress as well (Yoshioka et al., 2006). Another, as yet unidentified FtsH protease from pea thylakoids may degrade unassembled Rieske protein molecules that accumulated on the stromal side of thylakoid membrane (Ostersetzer and Adam, 1997). In its turn AtFtsH11 has been suggested to play a critical role in thermotolerance although a physiological substrate(s) for this enzyme has not been identified yet (Chen et al., 2006).

AtftsH2 (*var2*) and *AtftsH5* (*var1*) mutants show visible phenotypic alterations. Namely, *var2* exhibits a typical leaf-variegated phenotype derived from a formation of green sectors containing normal-appearing chloroplasts separated by white sectors containing undifferentiated plastids, arrested in thylakoid formation, substantially repressed in accumulation of nuclear-encoded photosynthetic genes (Chen et al., 2000; Kato et al., 2007; Takechi et al., 2000). As it was demonstrated that not only AtFtsH2 itself but also AtFtsH1/5 subunits accumulate in *var2* thylakoids to much lower level compared with wild type plants (Zaltsman et al., 2005a) it was concluded that the entire AtFtsH heterocomplex is involved in thylakoid formation besides being engaged in PSII repair. This notion is strongly supported by the observation that *AtftsH5* (*var1*) exhibits a typical, leaf-variegated phenotype as well, albeit with somewhat lower variegation degree with regard to *var2* (Martinez-Zapater, 1993; Sakamoto et al., 2002). The presence of abnormal plastids in white sectors of *var1* leaf, rich in highly vacuolated structures and clustered plastoglobuli but lacking well-developed thylakoids (Sakamoto et al., 2002) as well as a mounting evidence indicating that AtFtsH2/8 level is decreased significantly in *var1* (Yu et al., 2005) point unanimously to AtFtsH heterocomplex engagement in plastid development.

Despite these observations, virtually no one thylakoid bound apoprotein, besides PsbA has been identified as target for AtFtsH heterocomplex-mediated degradation, neither in non-stressing nor stressing conditions. Therefore, having borne in mind recent findings which show localization of FtsH hexamers in vicinity of PSII–LHCII supercomplexes in the grana (Yoshioka et al., 2010) we have sought to determine potential function of AtFtsH heterocomplex in stress mediated degradation of apoproteins of LHCII. In higher plants three types of genes (*Lhcb1–3*) code for respective LHCII apoproteins which bind chl*a*, chl*b* and xantophylls and form homo- or heterotrimers (for a review, see Ballottari et al., 2012). A dimeric PSII core can bind up to four LHCII trimers at two types of binding positions, labelled LHCII-S and LHCII-M (referring to strongly and moderately-bound LHCII, respectively) which form various PSII–LHCII supercomplexes by associating with monomeric peripheral antenna proteins (Boekema et al., 1999).

Four stresses have been selected for our studies, namely high irradiance, desiccation, cold and high salt. By using *var1-1* mutant, genetically identified by Martinez-Zapater (1993) we show hereby that AtFtsH heterocomplex repression leads to an inability to degrade Lhcb1 and Lhcb2 apoproteins following desiccation, high cold and high irradiance as well as an inability to degrade Lhcb3 apoprotein in response to high irradiance stress. Furthermore, we report on modulations in composition of LHCII trimers of BBY particles of wild type plants resulting from differential Lhcb1–3 disappearance behaviour following desiccation stress and show that LHCII of *var1-1* BBY particles resists the modulations.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana wild type (ecotype Columbia) and *var1-1* mutant plants were grown for four weeks in 42 mm Jiffy peat pellets on sphagnum peat moss and wood pulp (AgroWit, Przylep, Poland) in a growth chamber (NEMA, Netzschkau, Germany) under long day (16 h light/8 h dark) conditions with an irradiance set at 110 μmol photons m^{-2} s^{-1} at a constant temperature of 22 °C and 70% humidity.

Stress treatments

To test the changes in Lhcb1–3 levels the leaves detached from 4-week old plants (WT and *var1-1*) were exposed to four stresses: desiccation, high salt, cold and high irradiance. High salt stress was performed by floating the detached leaves on 400 mM NaCl solution. Desiccation was performed by placing leaves on pieces of Whatman 3MM paper. Cold stress was performed by floating detached leaves on water at 4 °C. High irradiance was performed by exposing the detached leaves floated on water to 800 μmol quanta m^{-2} s^{-1}. During the exposition of the leaves for the stress conditions the temperature (except for cold stress) and