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Deg/HtrA proteases as components of a network for photosystem II quality control in chloroplasts and cyanobacteria

Pitter F. Huesgen[1], Holger Schuhmann, Iwona Adamska*

Department of Plant Physiology and Biochemistry, University of Konstanz, Universitätsstrasse 10, D-78457 Konstanz, Germany

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Abstract

Organisms that perform oxygenic photosynthesis are subjected to photoinhibition of their photosynthetic function when exposed to excessive illumination. The main target of photoinhibition is the D1 protein in the reaction center of the photosystem II complex. Rapid degradation of photodamaged D1 protein and its replacement by a de novo synthesized functional copy represent an important repair mechanism crucial for cell survival under light stress conditions. This review summarizes the literature on the ATP-independent Deg/HtrA family of serine endopeptidases in cyanobacteria and chloroplasts of higher plants, and discusses their role in D1 protein degradation. We propose that Deg/HtrA proteases are part of a larger network of enzymes that ensure protein quality control, including photosystem II, in plants and cyanobacteria.

Keywords: Deg/HtrA protease; Chloroplast; Photosystem II; *Synechocystis* 6803

1. The family of Deg/HtrA proteases: a general overview

Deg/HtrA proteases are ATP-independent serine endopeptidases that are found in almost all domains of life, including Bacteria, Archaea and Eukarya. The first member from the Deg/HtrA family was discovered in *Escherichia coli* and named after the null mutant phenotypes DegP (for degradation of periplasmic proteins) [66] or, alternatively, HtrA (for the high temperature requirement A) [46]. Deg/HtrA proteases belong to the S1B subfamily of the clan PA according to the MEROPS nomenclature [55], and feature a catalytic domain of the trypsin type with His-Asp-Ser as a catalytic triad. Most Deg/HtrA family members contain C-terminally located PDZ domains, which regulate proteolytic activity [25,32,50,70] and are necessary for the formation of functional oligomeric complexes [25,34,59].

Deg/HtrA proteases are best studied in *E. coli* and mammals, where three (DegP/HtrA, DegQ/HhoA and DegS/HhoB) or five (HtrA1-4 and Tysnd1) of these enzymes are present, respectively. Structural studies showed that Deg/HtrA proteases form oligomeric complexes, with a trimer as the basic unit [41,44,70]. DegP/HtrA from *E. coli* forms a very compact, proteolytically inactive hexameric complex, where two homotrimers are stacked in a face-to-face manner [41]. Activation of DegP/HtrA, either allosterically by interaction of peptides with its PDZ1 domain [42,50] or a shift to higher temperatures [65], triggers an assembly into higher oligomeric structures. In solution, DegP/HtrA assembles around its substrates into large spherical 12- or 24-mers, composed of 4 or 8 homotrimers, respectively [33,43]. On lipid membranes, *E. coli* DegP/HtrA forms bowl-shaped structures, independent of the substrate, each with a 4-, 5-, or 6-fold symmetry with a trimer as the basic structural unit [61]. Also, human HtrA2/Omi [44] and *E. coli* DegS/HhoB [70] form homotrimers, but in contrast to *E. coli* DegP/HtrA, no further oligomerization of the basic trimers into higher order structures has been reported.

An intriguing feature of Deg/HtrA proteases is their functional versatility: DegP/HtrA in *E. coli* has been demonstrated to act as a chaperone or as a protease in a temperature-dependent

* Corresponding author. Tel.: +49 7531 88 2561; fax: +49 7531 88 3042.
E-mail addresses: pitter@interchange.ubc.ca (P.F. Huesgen), holger.schuhmann@uni-konstanz.de (H. Schuhmann), iwona.adamska@uni-konstanz.de (I. Adamska).
[1] Present address: Center for Blood Research, University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC, Canada V6T 1Z3.

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manner, providing quality control of protein folding in the periplasm [9,65,66]. *E. coli* DegS/HhoB, on the other hand, is a highly specialized enzyme with a single known physiological substrate. DegS/HhoB is activated in response to the accumulation of misfolded proteins in the periplasm, which is a first step in the signal transduction cascade that triggers a stress response in the cytoplasm [13].

Deg/HtrA proteases are involved in responses to various stress conditions. In many bacteria, including *E. coli*, protease DegP/HtrA is required for survival at elevated temperatures and participates in the response to oxidative stress (reviewed in [9,39,64]), while human HtrA2/Omi [69] and the yeast ortholog Nma111p [14] have been implicated in apoptosis. Human Deg/HtrA proteases have been shown to play critical roles in severe diseases such as Alzheimer's disease, age-related macular degeneration and several cancers (reviewed in [69]).

Less is known about Deg/HtrA proteases in photosynthetic organisms, including cyanobacteria and higher plants. The aim of this review was to provide a short summary on Deg/HtrA family members in cyanobacteria and their orthologs in chloroplasts of higher plants, with the main focus on their role in photosystem II (PSII) quality control.

2. Deg/HtrA proteases in cyanobacteria

All cyanobacteria investigated thus far contain between two and five Deg/HtrA proteases [26]. The only cyanobacterial orthologs studied in some detail are the three family members from *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803), named HtrA (*htrA, slr1204*), HhoA (*hhoA, sll1679*) and HhoB (*hhoB, sll1427*) in analogy to the *E. coli* enzymes [35]. In contrast to DegP/HhoA and DegQ/HhoA from *E. coli* that contain two PDZ domains, all three Deg/HtrA proteases from *Synechocystis* 6803 possess only one [26,39]. Sequence similarity suggested that the HtrA, HhoA and HhoB from *Synechocystis* 6803 are more closely related to each other than to proteases with the same names present in other organisms [26]. Inference of functions and mechanisms due to the same protease name should therefore be treated with great caution.

Proteome analysis found HtrA located in the outer membrane of *Synechocystis* 6803 [24] and HhoA in the periplasm, both in soluble [16] and in plasma membrane-bound [23] forms. The location of HhoB has not yet been experimentally proven, but this protease is predicted to target to the periplasm [39].

The Deg/HtrA proteases from *Synechocystis* 6803 have been proposed to be involved in response to light stress [17,62] and to play a role in the maintenance of the extracytoplasmic space, including soluble periplasmic proteins and intrinsic membrane proteins with periplasm-exposed loops, under heat and light stress [7,25]. However, physiological substrates of cyanobacterial Deg/HtrA proteases have not yet been identified.

Biochemical characterization of HhoA from *Synechocystis* 6803 showed that its proteolytic activity increased with temperature and basic pH and was stimulated by the addition of Mg^{+2} and Ca^{+2} [25]. The single PDZ domain of HhoA played a critical role in regulating protease activity and in the assembly of a homohexameric complex, in contrast to *E. coli* DegP/HtrA, where these functions were attributed to two PDZ domains [32,34]. Deletion of the PDZ domain strongly reduced, but did not abolish, proteolysis of sterically challenging substrates by *Synechocystis* 6803 HhoA protease [25].

3. Deg/HtrA proteases in chloroplasts of higher plants

The chloroplasts of green algae and higher plants have evolved from a cyanobacterial ancestor by endocytobiosis and still share many features with modern cyanobacteria, including the photosystem reaction center complexes [49]. Therefore, it is not surprising that the vast majority of proteases found in chloroplasts of green algae and higher plants are also derived from the prokaryotic ancestor [1,28,56]. An initial survey of the genome of *Arabidopsis thaliana* identified 13 Deg protease-encoding genes [1]. Later studies extended this number to sixteen genes [26,64]. Of these sixteen proteases, four are located in the chloroplasts. Deg1 (At3g27925), Deg5 (At4g18370) and Deg8 (At5g39830) of *A. thaliana* have been found to be attached to the luminal side of the thylakoid membrane [31,54,60], whereas Deg2 (At2g47940) is attached to the stromal side [20].

Deg1 had been identified as a housekeeping protease in the thylakoid lumen, degrading mistargeted and misfolded proteins in this compartment [8,31]. Plastocyanin and the 33 kDa protein of the oxygen evolving complex were identified as potential luminal substrates in vitro [8]. Size exclusion chromatography indicated that recombinant Deg1 was present in monomeric and homohexameric forms, both proteolytically active [8]. More recent studies revealed that Deg5, which lacks a PDZ domain, and Deg8 form a heterohexameric complex in a 1:1 stoichiometry [67]. Interestingly, recombinant Deg8 alone was proteolytically active, whereas recombinant Deg5 showed no enzymatic activity [67]. Studies on Deg2 demonstrated that this protease is peripherally associated with the non-appressed regions of the thylakoid membrane (e.g., stroma lamellae and grana margins and ends) and accumulates in response to high salt concentration, desiccation and light stress [20]. All three lumenal Deg proteases, Deg1, Deg5 and Deg8, and the stromal-side thylakoid-associated Deg2 were reported to participate in degradation of the photodamaged D1 protein at both sides of the thylakoid membrane [20,37,67,68].

4. PSII quality control under stress conditions

Organisms that perform oxygenic photosynthesis are subjected to inhibition of their photosynthetic functions when exposed to excessive light. This process is referred to as photoinhibition [4]. At ambient temperatures, the major target of photoinhibition is the PSII complex located in the thylakoid membrane and, in particular, its D1 reaction center protein. The D1 protein binds many of the cofactors involved in the primary and secondary electron flow and is therefore prone to irreversible oxidative damage by either reactive oxygen species or highly oxidizing species generated within PSII [4].