Family of Deg/Htr proteases in the cyanobacterium *Synechocystis* sp. PCC6803: investigations toward their expression and function

C Funk¹, K Haußühl¹,², I Adamska¹

¹Department of Biochemistry and Biophysics, Arrhenius Laboratories for Natural Sciences, Stockholm University, SE-10691 Stockholm, Sweden. Fax: +46 8 15 3679; Christine@dbb.su.se, Iwona@dbb.su.se
²MBP Cologne GmbH, Neurather Ring 1, DE-51063 Cologne, Germany. Fax: +492216470123; k.haussuehl@mpb-cologne.com

Keywords: cyanobacteria, D1 protein, Deg proteases, Htr/Hho, light regulation

Introduction

The members of the Deg/Htr family of serine endopeptidases are present in all living organisms investigated so far. While Prokaryota like *Escherichia coli* or cyanobacteria contain 3 members of the Deg/Htr family, in Eukaryota the number of Deg/Htr isomers varies between 2 (e.g. in human) and 13 (e.g. in *Arabidopsis thaliana*). It was shown recently that one member of the Deg/Htr family from *Arabidopsis*, called DegP2 performs the primary cleavage of the photodamaged D1 protein from the photosystem II (PSII) reaction center (Haußühl et al., 2001). The degradation of the D1 protein in higher plants and cyanobacteria occurs in at least two steps (reviewed in Aro et al., 1993). The primary cleavage of this protein takes place at the stromal loop connecting transmembrane α-helices D and E generating two proteolytic fragments. The secondary proteolysis involves a further degradation of these intermediates (Lindahl et al., 2000).

We investigated whether cyanobacterial homologues of the DegP2 protease, called HtrA or DegP (Cyanobase accession number slr1204), HhoA or DegQ (Cyanobase accession number sll1679) and HhoB or DegS (Cyanobase accession number sll1427) play a role during the degradation of the D1 protein in *Synechocystis* sp. PCC 6803. For this reason deletion mutants of HtrA, HhoA and HhoB proteases were generated and used for the investigation of the D1 protein degradation under different stress conditions. The results obtained with the HtrA mutant are shown in this proceeding.

We demonstrate here that the expression of the HtrA protease in *Synechocystis* wild type strain is strictly light dependent, which suggests its role during assembly/reassembly of the photosynthetic complexes. However, the HtrA deletion mutant had no clearly expressed phenotype and the D1 protein degradation occured with a kinetic similar to that measured for the wild type strain.

Materials and methods

*Growth of Synechocystis cultures and treatment conditions. Synechocystis* sp. PCC 6803 wild type and mutant cells were cultivated as described previously (Funk, 2000). To investigate the expression of HtrA protease during greening the PSI-less/chlL⁻ mutant (Wu and Vermaas, 1995) was grown for 7 days in the dark under light activated heterotrophic conditions (Anderson and McIntosh, 1991), than mutant cells were exposed to light (10 µmol m⁻² s⁻¹) and samples were collected at different stages of the greening process. For studying the role of
HtrA protease in the degradation of the D1 protein from the PSII the HtrA deletion mutant was exposed to light stress (1000 µmol m⁻² s⁻¹) or heat shock (42°C) conditions for 24 hours and samples were collected at various stages of the stress treatment as mentioned in the figure.

Functional deletion of htrA gene. The htra gene in Synechocystis is composed of 1359 bp and a 278 bp fragment of this gene was deleted using two BstEII restriction sites. The deleted fragment was replaced by a 1.0 kb kanamycin-resistance marker placed between 777 bp upstream and 303 bp downstream in the original HtrA gene. Transformants were allowed to segregate at 50 µmol m⁻² s⁻¹ as described previously (Funk, 2000).

RNA and protein analysis. Isolation of RNA and Northern blot conditions were performed as described (Funk and Vermaas, 1999). Thylakoids from Synechocystis wild type and the HtrA deletion mutant were isolated, proteins separated by SDS-PAGE and analyzed by immunoblotting as reported previously (Haußühl et al., 2001) using a polyclonal antibody raised against the D1 protein.

Results and discussion

It was reported that the heat shock treatment increases the expression of three members of the Deg/Htr protease family from Escherichia coli (reviewed in Gottesman, 1996), the HtrA2 protease from human (Gray et al., 2000) and the chloroplast DegP1 protease from Arabidopsis (Itzhaki et al., 1998). In contrast, the DegP2 protease from Arabidopsis was down-regulated in response to heat shock treatment (Haußühl et al., 2001). Our preliminary results revealed that the expression of the HtrA gene in Synechocystis is not significantly influenced by the heat shock treatment (not shown). Since Synechocystis grows photoautotrophically we examined whether light plays a role in regulation of the htrA gene expression.

![Fig. 1. Regulation of the htrA gene expression by light.](image)

It was shown that the expression of DegP2 protease in Arabidopsis is stimulated by high intensity light, high salt concentration and desiccation (Haußühl et al., 2001). To investigate whether the expression of the htrA gene in cyanobacteria is influenced by light, greening experiments were performed. The PSI-less/chL⁻ mutant (Wu and Vermaas, 1995), which can synthesize chlorophyll only in light was used for our studies. In “etiolated” mutant cells, no
htrA transcripts were detected by Northern blotting (Fig. 1). Exposure of mutant cells to light resulted in a rapid induction of htrA transcripts and the amount of these transcripts increased with the time of illumination. In green mutant cultures the htrA transcripts decreased again to an undetectable level (Fig. 1). However, when light-grown Synechocystis cells were exposed to light stress for several hours the htrA transcript level increased significantly (not shown). Such a light dependent expression of the htrA suggests that this protease may play a role during the assembly/disassembly of the photosynthetic complexes or be involved in the proteolysis of the photodamaged protein subunits.

It was shown that the DegP2 protease in Arabidopsis performs the primary cleavage of the photodamaged D1 protein from the PSII reaction center. To investigate the role of htrA protease in the degradation of the D1 protein we constructed a deletion mutant, in which the htrA gene was interrupted by the insertion of a kanamycin resistance cassette (Fig. 2A). To prove the segregation of wild type and mutant genotypes with respect to the htrA locus sizes of the PCR amplified htrA genes from wild type and mutant strains were analyzed on agarose gel. The results confirmed that the segregation of the Δ htrA mutant was completed (Fig. 2B). The htrA deletion mutant showed neither a specific phenotype nor significant changes of photoautotrophic growth at 50 µmol m⁻² s⁻¹ (not shown).

To assay potential changes in the D1 degradation the htrA deletion mutant and wild type cultures were exposed to light stress or heat shock conditions and kinetics of the D1 degradation were assayed by immunoblotting. These stress conditions are known to induce damage to the D1 protein and thus enhance its degradation rate (reviewed in Aro et al., 1993). The results revealed (Fig. 2C) that the exposure of wild type and mutant cultures to light stress resulted in the diminished level of the D1 protein that was visible already after 4 hours of the stress treatment. After 24 hours of light stress exposure only traces of the D1 protein were detected in both cultures. This effect was less drastic when wild type and mutant cultures were subjected to the heat shock treatment. Only prolonged exposure to high temperature resulted in the complete loss of the D1 protein. However, under both stress conditions the degradation of the D1 protein did not differ significantly between the htrA deletion mutant and the wild type strain. Thus, we conclude that either other Deg/Htr isomers perform the D1 protein degradation or that other members of this family can substitute for each other in their physiological function. The latter aspect is under current investigation.

Acknowledgements

This work was supported by research grants from the Swedish Natural Research Council, the Swedish Strategic Foundation, the Carl Tryggers Foundation and the Ekströms Donation from the Stockholm University.
Fig. 2. Degradation of the D1 protein in the HtrA deletion mutant of *Synechocystis*. (A) Interruption of the htrA gene by insertion of a kanamycin resistance cassette. (B) Segregation of wild type and mutant genotypes with respect to the HtrA locus tested by the PCR amplification. (C) Kinetics of the D1 protein degradation assayed by immunoblotting under light stress (HL) and heat shock (HS) conditions.

References