

## The role of the FtsH protease in the repair of Photosystem II after damage by UV-B radiation in *Synechocystis* 6803

Otilia Cheregi<sup>1</sup>, Cosmin Sicora<sup>1</sup>, Peter B Kos<sup>1</sup>, Peter J Nixon<sup>2</sup>, Imre Vass<sup>1\*</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary, <sup>2</sup>Department of Biological Sciences, Imperial College London, Biochemistry Building, South Kensington, London, UK

**ABSTRACT** We have investigated the repair mechanism of UV-damaged PSII in *Synechocystis* 6803 cells, in relation to the role of the FtsH protease. FtsH is an ATP- dependent metalloprotease that belongs to the AAA- (ATP-ase associated with diverse cellular activities) protein family. One of the homologues is encoded by the slr0228 gene whose transcript level is induced 16 fold by UV-B radiation. The UV-B induced loss of oxygen evolving activity was accelerated in the  $\Delta$ FtsH mutant, which lacks the FtsH protease due to the deletion of the slr0228 gene. In the presence of the protein synthesis inhibitor lincomycin, the rate of UV-B induced oxygen inhibition in the  $\Delta$ FtsH mutant show the same kinetics as in the absence of antibiotic. In addition, the mutation completely abolished the restoration of oxygen evolution in visible light following UV-B treatment. These data demonstrate that PSII repair is blocked in the absence of the FtsH protease both under UV illumination and in visible light following the UV-B treatment.

Acta Biol Szeged 49(1-2):199-201 (2005)

### KEY WORDS

PSII repair  
UV-B radiation  
FtsH protease  
oxygen evolution  
lincomycin

The most sensitive target of UV-B radiation is the light energy-converting photosystem II complex of the photosynthetic apparatus. The primary target site of UV-B irradiation has been identified at the donor side of PSII, at the level of the Mn cluster of water oxidation (Vass et al. 1996). UV radiation affects also the Tyr-Z and Tyr-D electron donors (Vass et al. 1996) as well as the Q<sub>A</sub> and Q<sub>B</sub> quinone electron acceptors (Vass 1999).

An important consequence of the UV-B irradiation is the degradation of the D1 and D2 subunits of PSII reaction centre (Greenberg et al. 1989; Melis et al. 1992).

The ability of plants to avoid and/or repair UV-B induced damages determines their capability to tolerate the adverse effects of UV-B irradiation. In intact photosynthetic organism the structure and the function of photodamaged PSII centers can be repaired. The critical step of this repair process is the novo synthesis of the damaged D1 and D2 subunits followed by the reassembly and reactivation of PSII complex (Aro et al. 1993; Sass et al. 1997). The repair process is preceded by proteolytic removal of the damaged D1 protein; however, the identities of the proteases involved remain unknown despite intense research efforts.

The FtsH protease has been shown to play an important role in the turnover of the D1 protein when induced by visible light both in higher plants and in cyanobacteria (Bailey S et al. 2002; Silva P et al. 2003).

Inactivation of one of the four FtsH homologues- slr0228- showed altered pigmentation due to a 60% decrease in the content of the PSI reaction centre. Here we report the analysis

of PSII repair in a specific FtsH mutant of *Synechocystis* PCC6803, slr0228:: $\Omega$ .

### Materials and Methods

*Synechocystis* sp. PCC 6803 cells were routinely grown in BG-11 medium in a rotary shaker at 30°C under a 5% CO<sub>2</sub>-enriched atmosphere. The  $\Delta$ FtsH mutant was constructed by interrupting the slr0228 gene with a chloramphenicol resistance cassette as described by Silva et al. UV-B irradiation was performed in open, square glass containers in which the cell suspension of 6.5  $\mu$ g Chl ml<sup>-1</sup> formed a layer of 1 cm height, with continuous stirring. A Vilbert-Lourmat VL-215M lamp was used as UV-B light source, with maximal emission at 312 nm, in combination with an 0.1 mm cellulose acetate filter (Clarfoil, Courtaluds Chemicals, UK) in order to screen out any UV-C contribution. The UV-B intensity was 4.8 Wm<sup>-2</sup> ( $\approx$ 13  $\mu$ Em<sup>-2</sup>s<sup>-1</sup>) at the surface of the cell suspension. For the recovery experiments, visible light was produced by an array of halogen spot lamps in the 40-50  $\mu$ E m<sup>-2</sup>s<sup>-1</sup> intensity range.

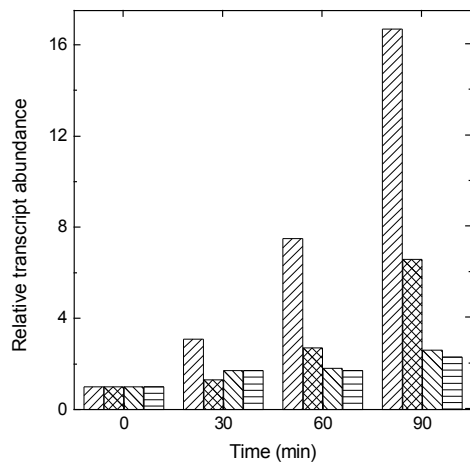
UV- induced changes in the transcripts level of the FtsH homologues genes were detected by quantitative RT PCR.

Light-saturated steady-state rate of oxygen evolution was measured using a Hansatech DW2 O<sub>2</sub> electrode at a light intensity of 1000  $\mu$ Em<sup>-2</sup>s<sup>-1</sup> in the presence of 0.5 mM 2,5-dimethyl-p-benzoquinone as electron acceptor.

### Results

The cyanobacterium *Synechocystis* 6803 has four homologues of the membrane bound ATP-dependent FtsH protease encoded by the slr0228, slr1390, slr1604 and slr1463 genes

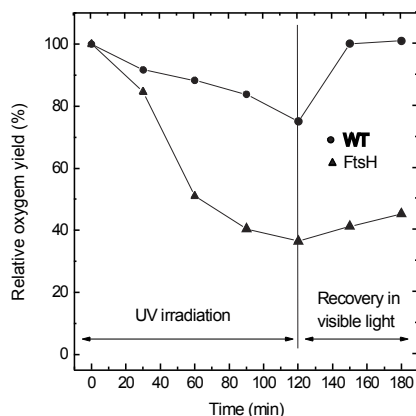
\*Corresponding author. E-mail: imre@nucleus.szbk.u-szeged.hu



**Figure 1.** UV-B induced expression of the FtsH homologue genes. The transcript levels were detected by quantitative RT PCR for the *slr0228* (right hatch), *slr1604* (crossed hatch), *slr1390* (left hatch), *sl11436* (horizontal hatch) genes.

(Sokolenko et al. 2002). A RT PCR experiment in our lab showed that all four genes were induced more than 2-fold after 90 min UV-B exposure with the most dramatic effect on the *slr0228* gene which was induced 16 fold (Fig. 1).

To determine whether the loss of FtsH gene (*slr0228*) impaired the ability of the cells to repair damaged PSII, PSII activity measured as light-saturated rates of oxygen evolution, was monitored in cells as a function of exposure to UV-B radiation and subsequent recovery in visible light, either in the presence or in the absence of lincomycin. Lincomycin is an antibiotic that blocks de novo protein synthesis and thus



**Figure 2A.** UV-B induced loss, and recovery of oxygen evolution. The time course of oxygen evolution was followed in the WT (circles) and  $\Delta$ FtsH cells (up triangles) during exposure to UV-B light and subsequent recovery in low intensity ( $40 \mu\text{Em}^{-2}\text{s}^{-1}$ ) visible light.

the repair of PSII.

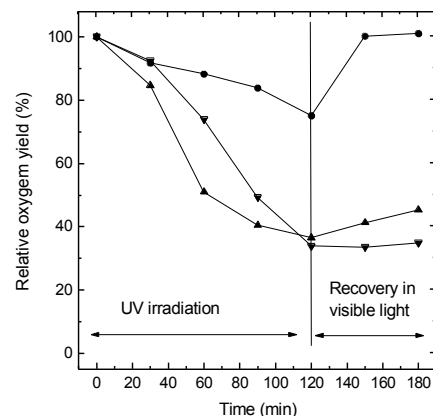
Illumination with UV-B induced a 20% loss of oxygen activity in the WT and a 60% loss in the FtsH mutant. During the one hour recovery the WT fully recovered while in the mutant a very weak recovery process was measured. Under these conditions, in the mutant the PSII repair was unable to compensate the rate of damage (Fig. 2A).

The loss of oxygen evolution in the  $\Delta$ FtsH mutant has practically the same kinetics as measured in the WT in the presence of protein synthesis inhibitor lincomycin (Fig. 2B). The fast rate of damage obtained in the presence of lincomycin represent the “true rate” of PSII inactivation in the absence of any repair process. The slower rates of PSII inactivation in the absence of lincomycin represent the balance between UV-B inactivation and its continuous repair. The same pattern of oxygen evolution inhibition in the mutant and wild type with lincomycin, proves that in the absence of FtsH protease there is no protein synthesis. It is known that the UV-B irradiation damages the D1 protein and enhance the turnover of this subunit in vivo. If there is no repair process of the damaged D1 the PSII function cannot be rehabilitated and this is obvious in the loss of oxygen activity. The results clearly show that de novo protein synthesis is needed to restore PSII activity following UV-B induced damage.

## Conclusions

Our data demonstrate that the FtsH protease is needed for the repair of PSII during and after UV-B treatment in *Synechocystis* 6803 cells.

Considering that repair of PSII activity requires de novo synthesis of the D1 reaction center protein (Sass et al. 1997), we conclude that in the  $\Delta$ FtsH strain the degradation and synthesis of the D1 protein is blocked.



**Figure 2B.** The time course of oxygen evolution is also shown in the presence of lincomycin for the WT cells (down triangles).

## Acknowledgments

This work has been supported in by a grant from the Hungarian Granting Agency OTKA (T034321).

## References

- Aro E-M, Virgin I, Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochim Biophys Acta* 1143:113-134.
- Bailey S, Thompson E, Nixon PJ, Horton P, Mullineaux CW, Robinson C, Mann NH (2002) A Critical Role for the Var2 FtsH Homologue of *Arabidopsis thaliana* in the Photosystem II Repair Cycle in Vivo. *J Biol Chem* 277:2006-2011.
- Friso G, Spetea C, Giacometti GM, Vass I, Barbato R (1994) Degradation of photosystem II reaction center D1-protein induced by UVB irradiation in isolated thylakoids. Identification and characterization of C- and N-terminal breakdown products. *Biochim Biophys Acta* 1184:78-84.
- Greenberg BM, Gaba V, Canaani O, Malkin S, Matoo AK, Edelman M (1989) Separate photosensitizers mediate degradation of the 32-kDa Photosystem II reaction centre protein in visible and UV spectral regions. *Proc Natl Acad Sci USA* 86:6617-6620.
- Sass L, Spetea C, Máté Z, Nagy F, Vass I (1997) Repair of UV-B induced damage of Photosystem II via de novo synthesis of the D1 and D2 reaction centre subunits in *Synechocystis* sp. PCC 6803. *Photosynth Res* 54:55-62.
- Silva P, Thompson E, Bailey S, Kruse O, Mullineaux CW, Robinson C, Mann NH, Nixon PJ (2003) FtsH Is Involved in the Early Stages of Repair of Photosystem II in *Synechocystis* sp PCC 6803. *Plant Cell* 15:2152-2164.
- Sokolenko A, Pojidaeva E, Zinchenko V, Panichkin V, Glaser VM, Herrmann RG, Shestakov SV (2002) The gene complement for proteolysis in the cyanobacterium *Synechocystis* sp. PCC 6803 and *Arabidopsis thaliana* chloroplasts. *Curr Genet* 41:291-310.
- Vass I, Sass L, Spetea C, Bakou A, Ghanotakis D, Petrouleas V (1996) UV-B induced inhibition of photosystem II electron transport studied by EPR and chlorophyll fluorescence. Impairment of donor and acceptor side components. *Biochemistry* 35:8964-8973.
- Vass I, Kirilovsky D and Etienne A-L (1999) UV-B radiation- induced donor- and acceptor side modifications of Photosystem II in the cyanobacterium *Synechocystis* sp.PCC 6803. *Biochemistry* 38:12786-12794.