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ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 820–828
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

The role of the FtsH and Deg proteases in the repair of UV-B radiation-damaged Photosystem II in the cyanobacterium *Synechocystis* PCC 6803

Otilia Cheregi [a], Cosmin Sicora [a,1], Peter B. Kós [a], Myles Barker [b], Peter J. Nixon [b], Imre Vass [a,*]

[a] *Institute of Plant Biology, Biological Research Center, Szeged, Hungary*
[b] *Division of Biology, Imperial College London, South Kensington campus, London, SW7 2AZ, UK*

Received 29 September 2006; received in revised form 13 November 2006; accepted 14 November 2006
Available online 5 December 2006

Abstract

The photosystem two (PSII) complex found in oxygenic photosynthetic organisms is susceptible to damage by UV-B irradiation and undergoes repair *in vivo* to maintain activity. Until now there has been little information on the identity of the enzymes involved in repair. In the present study we have investigated the involvement of the FtsH and Deg protease families in the degradation of UV-B-damaged PSII reaction center subunits, D1 and D2, in the cyanobacterium *Synechocystis* 6803. PSII activity in a ΔFtsH (slr0228) strain, with an inactivated slr0228 gene, showed increased sensitivity to UV-B radiation and impaired recovery of activity in visible light after UV-B exposure. In contrast, in ΔDeg-G cells, in which all the three *deg* genes were inactivated, the damage and recovery kinetics were the same as in the WT. Immunoblotting showed that the loss of both the D1 and D2 proteins was retarded in ΔFtsH (slr0228) during UV-B exposure, and the extent of their restoration during the recovery period was decreased relative to the WT. However, in the ΔDeg-G cells the damage and recovery kinetics of D1 and D2 were the same as in the WT. These data demonstrate a key role of FtsH (slr0228), but not the Deg proteases, for the repair of PS II during and following UV-B radiation at the step of degrading both of the UV-B damaged D1 and D2 reaction center subunits.

Keywords: Photosystem II; D1-protein; D2-protein; FtsH protease; UV-B damage

1. Introduction

Ultraviolet-B (UV-B, 280–320 nm) radiation is a harmful component of sunlight that damages all forms of life including photosynthetic microbial organisms [1,2]. In plants and cyanobacteria a well-documented effect is the inhibition of photosynthesis leading to decreased oxygen evolution and CO_2 fixation [3–5]. Within the photosynthetic apparatus the most UV-B sensitive component is the light-energy converting Photosystem II (PSII) complex, whose electron transport capacity is inhibited and protein structure is damaged (for reviews see [6,7]). Inside PSII, the primary target of UV-B radiation is the Mn cluster of the water-oxidizing complex [8–10], with additional effects at the Tyr-Z and Tyr-D electron donors [11], and the Q_A and Q_B quinone electron acceptors [7,9,12]. UV-B has negative effects not only on electron transport of PSII but also on the key reaction center proteins, D1 and D2, which are degraded under UV-B irradiation [12–15].

To avoid permanent inhibition of PSII function due to the loss of the D1/D2 heterodimer, a tightly regulated repair process occurs in the thylakoid membranes of cyanobacteria and plants to replace the damaged proteins with new, fully functional copies [16–19]. The main steps of the PSII repair cycle are thought to involve: (i) a structural change to signal the need for damaged subunits to be removed after photodamage; (ii) monomerization and partial disassembly of the dimeric PSII complex to allow access to the damaged protein subunits; (iii) degradation of the damaged D1 and D2 subunits and synchronized replacement by newly synthesized copies, and (iv) reassembly of the extrinsic proteins and the Ca–Mn cluster

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSII, photosystem II; WOC, water oxidation complex

* Corresponding author. Fax: +36 62 433 434.
E-mail address: imre@brc.hu (I. Vass).
[1] Present address: Biology Department, Turku University, Turku, Finland.

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doi:10.1016/j.bbabio.2006.11.016

to the complex, which can occur only after C-terminal processing of the D1 subunit by the CtpA protease. In contrast to photodamage by visible light, where mainly the D1 subunit is damaged and repaired, UV-B radiation damages the D1 and D2 proteins to almost the same extent and the repair process includes *de novo* synthesis of both subunits [15].

As yet the proteases involved in PSII repair following UV-B damage have not been identified and it still remains unclear the extent to which non-enzymatic reactions are involved in protein degradation. *In vitro* studies using isolated PSII membrane fragments or detergent-solubilized PSII complexes indicated the formation of a 20-kDa C-terminal D1 fragment via a non-enzymatic mechanism, which would place the primary cleavage site of D1 to the middle of the second transmembrane helix [14]. However, this fragment did not accumulate to a significant amount, and the D1 protein was degraded also under conditions when the 20-kDa C-terminal fragment could not be observed. The degradation of D2 also appeared to be independent of protease activity in isolated PSII reaction center complexes [20]. Small amount of the 20 kDa D1 fragment was also observed when intact leaves were illuminated by UV-B. However, this fragment was unstable in visible light pointing to the possibility that protease activity is involved in the final step of the degradation process [21].

In the case of visible-light damage the FtsH and Deg proteases have both been implicated in PSII repair *in vivo*. There are four predicted FtsH proteases in the cyanobacterium *Synechocystis* 6803, designated slr0228, slr1604, slr1390 and sll1463. Insertional mutagenesis experiments revealed that two genes were absolutely required for cell viability (slr1390 and slr1604), one had no obvious phenotype (sll1463), and the fourth (slr0228) caused an altered pigmentation due to a 60% decrease in the content of PSI [22]. FtsH (slr0228) mutants were subsequently found to show visible-light sensitive growth, impaired PSII repair and retardation of D1 degradation *in vivo* [23]. The persistence of full-length D1 protein in the FtsH (slr0228) mutant, the co-purification of slr0228 with His-tagged PSII [23], and the exclusion of the functional role of other cyanobacterial proteases in the cleavage of damaged D1 protein has led to a general model for PSII repair in which FtsH complexes alone are able to degrade visible-light damaged D1 [19]. FtsH protease activity has also been associated with the degradation of oxidatively damaged D1 protein *in vivo* in higher plants [24,25].

In contrast an alternative view emphasizes the involvement of the DegP/HtrA or Deg proteases in PSII repair and D1 degradation following visible light stress, both in chloroplasts [26] and cyanobacteria [27]. In *Synechocystis* 6803 there are 3 members of the Deg family of proteases: HtrA (slr1204), HhoA (sll1679) and HhoB (sll1427) [28]. In the model of Huesgen et al. [27], which is partially supported by *in vitro* data [29], D1 is proposed to be cleaved in periplasmic-exposed loops by the HhoA protease. However, it has been recently reported that although the Deg proteases are required for photo-tolerance, they are not involved in D1 turnover following visible-light stress [19,30]. Whether FtsH and Deg proteases have a role in the response to UV-B damage is unclear.

Recent microarray data have indicated that UV-B radiation strongly induces the transcript levels of the *ftsH* (*slr0228)* gene in *Synechocystis* 6803 [31,32]. This observation points to the possibility that the FtsH (slr0228) protease could be involved in the repair of UV-damaged PSII complex similarly to its previously documented role in visible light stress [22].

Here we have studied the effect of inactivating the *slr0228* gene and the 3 *deg* genes of *Synechocystis* 6803 on PSII repair during and after UV-B damage. Our results show that in the Deg triple mutant, D1 and D2 degradation proceeds at the same rate as in the WT during UV-B radiation, and the efficiency of PSII repair is unaffected. However, in the ΔFtsH (slr0228) mutant PSII repair is largely abolished, and D1 and D2 protein degradation is retarded. Our work demonstrates the participation of the FtsH (slr0228), but not of the Deg proteases, in the repair of UV-damaged PSII, and importantly, that removal of damaged PSII subunits is mainly enzymatic *in vivo*.

2. Materials and methods

2.1. Synechocystis strains and growth conditions

The glucose-tolerant strain of *Synechocystis* sp. PCC 6803 was used to construct the mutants [33]. Cells were routinely grown in BG-11 medium in a rotary shaker at 30 °C under a 5% CO_2-enriched atmosphere and 40 μE/m^{-2} s^{-1} light intensity. The ΔFtsH (slr0228) mutant was constructed by interrupting the *slr0228* gene with a chloramphenicol-resistance cassette [23]. The three DegP/HtrA genes were inactivated stepwise using the plasmids described earlier [34]: first *hhoA*, then *hhoB* to generate the *hhoAhhoB* double mutant and finally the *htrA* to give the ΔDeg-G triple mutant [30]. The genes were interrupted by chloramphenicol, erythromycin and kanamycin-resistance cassettes, respectively.

2.2. UV-B treatment

UV-B radiation was performed in open, square glasses in which 100 ml cell culture of 6.5 μg Chla/ml formed 1 cm layer height, maintained in suspension by magnetic agitation. UV-B light was provided by a Vilbert–Lourmat lamp, with maximum emission at 312 nm, in combination with 0.1 mm cellulose acetate filter (Clarfoil, Courtalouds Chemicals, UK) yielding an intensity of 12 μE m^{-2} s^{-1} at sample surface. In some cases, a protein–synthesis inhibitor, either lincomycin (at 300 μg/ml) or spectinomycin (at 200 μg/ml), was added to the cell culture. For the recovery experiments, visible light was produced by an array of halogen spot lamps in the 40–50 μE m^{-2} s^{-1} intensity ranges.

2.3. Oxygen evolution measurements

PSII activity was assessed by measuring the light-saturated rate of oxygen evolution from whole cells, in the presence of 0.5 mM 2,5-dimethyl-p-benzoquinone as electron acceptor, using a Hansatech DW2 O_2 electrode. Usually, 1 ml of cells at 6.5 μg Chl a/ml was used in each measurement.

2.4. Fluorescence measurements

Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured by a double-modulation fluorometer (P.S.I. Instruments, Brno, Czech Republic) [35], in the 150 μs to 100 s time range, in samples which were dark adapted for 3 min prior to measurements, as described by [9].

2.5. Thylakoid preparation and protein analysis

Thylakoid membranes were prepared by breakage of the cells with glass beads (150–200 μm in diameter) at 4 °C followed by differential centrifugation