ABSTRACT OF THE THESIS

INVOLVEMENT OF PROTEASES, CYCLIC NUCLEOTIDES AND SMALL HEAT SHOCK PROTEINS IN PSII REPAIR IN *SYNECHOCYSTIS* sp. PCC6803

PhD Thesis

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INTRODUCTION

Cyanobacteria, the most widespread and abundant oxygenic photosynthetic prokaryotes, are exposed to various types of environmental stresses. With the recent thinning of the ozon layer, the UV-B component of solar radiation is of particular importance. The main consequences of UV-B induced damages are the inactivation of electron transport chain (Vass, 1999) and the degradation of the D1 and D2 reaction center proteins (Friso et al. 1994; Friso et al. 1995). Photosynthetic organisms can cope with the UV-B induced damages because of an efficient PSII repair cycle that replaces the damaged subunits. The events of the repair cycle are highly coordinated are require monomerization of PSII dimers, proteolytic degradation of damaged subunits, the ribosomal protein synthesizing machinery, reinsertion of newly synthesized proteins into the thylakoid membrane, reassembly of PSII monomers and photoactivation, and finally dimerization of monomers into oxygen-evolving PSII centers (Aro et al. 1993;Nixon et al. 2005).

In this thesis, several aspects concerning the PSII repair cycle following UV-B induced damage have been studied.

The degradation of D1 and D2 proteins is known to be a proteolytic process but the identity of proteases involved is a field of intense research. FtsH (*F*ilamentation *t*emperature-*s*ensitive) proteases are localized in PSII complexes, with the protease domain oriented toward the cytoplasmic side of the thylakoid membrane (Silva et al. 2003). Inactivation of two of the four *ftsH* genes in *Synechocystis* PCC6803 proved to be lethal, one had no obvious phenotype and the mutation of *slr0228* caused an increased sensitivity to light and impaired PSII repair (Silva et al. 2003). The *slr0228* gene in *Synechocystis* PCC6803 is a very close homologue of the *var2* of *Arabidopsis* which is involved in PSII repair and chloroplast biogenesis (Bailey et al. 2002). The Deg family of proteases has three members in *Synechocystis* PCC6803 (Sokolenko

et al. 2002), which are encoded by homologues of *deg* genes of higher plants. Their role in PSII repair has been a subject of intense research leading to partly contradictory data (Lindahl et al. 2000; Kanervo et al. 2003; Huesgen et al. 2005; Barker et al. 2006). Here we have studied the effect of inactivating the *slr0228* gene and the three *deg* genes of *Synechocystis* PCC6803 on PSII repair during and after UV-B damage.

Although the effects and consequences of UV-B radiation are known in detail the mechanisms for sensing and responding to UV-B are largely unknown. The cyclic nucleotides cAMP (3',5'- cyclic adenosine monophosphate) and cGMP (3',5'- cyclic guanosine monophosphate) are typical second messengers, intracellular receptors of extracellular signals and their discovery was awarded with a Nobel prize. The cellular level of cyclic nucleotides is an equilibrium between the activities of adenylyl-, guanylyl-cyclases (which catalyze the synthesis of cAMP and cGMP from ATP, respectively) and cyclic nucleotide phosphodiesterases (which catalyze the degradation of the two cyclic nucleotides). The open reading frames *slr2100* and *slr1614* are proposed putative cNMP phosphodiesterases because they each carry a HD domain (de Alda et al. 2000b). Our work aimed to investigate the regulatory network by which *Synechocystis* PCC6803 senses UV-B, through the analysis of a cyclic nucleotide phosphodiesterase mutant, Δ*slr2100*.

The small heat shock protein 17 (Hsp17) confers thermal resistance to PSII apparatus during heat shock (Nakamoto et al. 2000). *Synechocystis* PCC6803 has only one small heat shock protein (sHSP) which is induced by many stress conditions; the newly synthesized proteins are associated with the thylakoid membrane (Horvath et al. 1998). A glutamine to arginine residue change in the structure of Hsp17 induces a strong association of mutant protein with the lipids of the thylakoid membrane. We wanted to verify if this interaction modifies the activity of PSII under UV-B stress conditions.

AIMS OF THE STUDY

In our experiments we were aiming to identify factors and their contribution to the repair of UV-B damaged PSII reaction center in the cyanobacterium *Synechocystis* PCC6803. The particular goals of our work were:

1. To find which protease is involved in the degradation of the UV-B damaged D1 and D2 proteins of the PSII reaction center complex. Therefore, we took advantage of a series of mutants for two families of proteases: Deg and FtsH.
2. The open reading frame *slr2100* is a proposed cNMP phosphodiesterase because it carries a HD domain. The questions we addressed were: what is the *in vivo* function of this gene? Do cyclic nucleotides play a role in the signaling pathways of PSII repair?
3. Due to the preferential and selective association of the Q16R-Hsp17 protein with the thylakoid membrane after heat shock our goal was to verify if this event confers increased resistance to PSII damage or facilitates the recovery/repair from UV-B damage.

MATERIALS AND METHODS

- *Synechocystis* growth conditions
- Thylakoid isolation
- Chlorophyll content determination
- Visible and ultraviolet light treatment
- Measurement of photosystem II electron transport activity
- Gel electrophoresis and immunoblotting

RESULTS AND DISCUSSION

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* PCC6803. PSII activity in a ΔFtsH/*slr0228* strain, measured as the rate of oxygen evolution, showed increased sensitivity to UV-B radiation and impaired recovery of activity in visible light after UV-B exposure. In contrast, in Δ*deg* cells, in which all the three *deg* genes were inactivated, the damage and recovery kinetics were the same as in WT. Immunoblotting showed that the loss of both the D1 and D2 protein 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 cells the damage and recovery kinetics of D1 and D2 were the same as in the WT. Moreover, we have not detected any fragments of the D1 or D2 proteins in the absence of FtsH or Deg proteases which indicates that degradation pathways involving non-proteolytic D1 and D2 fragment formation are not significant in intact *Synechocystis* PCC6803 cells.

To get information about the possible implication of *slr2100* gene, a putative phosphodiesterase, in the UV-B transduction pathway we measured the activity of the mutant cells in the conditions of high light and UV-B stress. The consequences of high light treatment on the PSII activity, measured as oxygen evolution, were the same in the WT and Δ*slr2100* mutant. In contrast, differences were observed in the response to a UV-B stress. A possible explanation of the increased UV-B sensitivity of the Δ*slr2100* mutant is that the mutation has an effect on the cascade of events required for the repair of the UV-B damaged PSII centers, which is known to require *de novo* protein synthesis. Indeed, the decreased amount of D1 protein in the thylakoids of the mutant strain exposed to UV-B compared to that of the wild type demonstrates

that the degradation part of the repair cycle is not affected by the lack of the *slr2100* gene.

Due to the preferential and selective association of the Q16R-Hsp17 protein with the thylakoid membrane after heat shock our goal was to verify if this event confers increased resistance to PSII damage or facilitates the recovery/repair from UV-B damage. Measurements of flash-induced chlorophyll fluorescence in the mutant cells revealed a specific modification of the thylakoid embedded PSII: acceleration of plastoquinone binding to the Q_B site. The modification on the Q_B binding site was further investigated by measuring the PSII activities in the presence of various quinones, in the WT and Q16R-Hsp17 mutant, before and after the heat shock. The number of PSII active centers is not affected by the 3 hours treatment at 42°C but the electron transfer efficiencies of these centers, measured with three different quinones acceptors, are different for the WT and Q16R-Hsp17 mutant. The Q16R-Hsp17 mutant shows reduced UV-B damage of PSII activity in comparison with the WT. The association of Q16R-Hsp17 protein with the thylakoid membrane and the preferential interaction with the SQDG lipids could confer UV-B protection by facilitating the PSII repair cycle at one or more levels: D1 degradation, resynthesis/reinsertion into the membrane, PSII monomerization/dimerization.

CONCLUSIONS

1. Our work demonstrates the participation of the FtsH/Slr0228 protease in the repair of UV-B damaged PSII reaction center. The FtsH protease is involved in the *in vivo* proteolytic removal of both D1 and D2 proteins of the PSII complex. Deg proteases do not seem to have a role in PSII repair following UV-B induced damage either in D1 and D2 proteins proteolysis (Cheregi et al. 2007).

2. We have demonstrated that when the *slr2100* gene is inactivated, the repair of UV-B damaged PSII is retarded. In the ΔSlr2100 mutant the level of cGMP is unregulated and this affects the adaptation of PSII apparatus to UV-B stress. This work points to the participation of the Slr2100 in the regulatory network by which *Synechocystis* PCC6803 senses UV-B light (Cadoret et al. 2005)

3. We have shown that the Q16R-Hsp17 mutant, with an enhanced lipid-mediated thylakoid membrane interaction, is able to protect PSII functions under UV-B photoinhibitory conditions. The protection of PSII function is exerted at the level of PSII repair through a facilitated D1 repair cycle. Besides this effect, the Q16R mutation in the HSP17 modifies the acceptor side of the PSII complex at the level of Q_B (Balogi et al. submitted).

LIST OF PUBLICATIONS

(* publications directly related to the subject of the Thesis)

1. * Cadoret, J.-C., Rousseau, B., Perewoska, I., Sicora, C., **Cheregi, O.**, Vass, I. and Houmard, J. (2005) „ Cyclic nucleotides, the photosynthetic apparatus and response to a UV-B stress in the cyanobacterium *Synechocystis* 6803" *J. Biol. Chem.* 280: 33935-33944. **IF:5.854**

2. * **Cheregi, O.**, Sicora, C., Kós. P.B., Barker, M., Nixon, P. and Vass, I. (2007) The role of the FtsH and Deg proteases in the repair of UV-B radiation-damaged Photosystem II in the cyanobacterium *Synechocystis* PCC 6803, *Biochim. Biophys. Acta* 1767:820-828. **IF:4.302**

3. Vass, I., Cser, K. and **Cheregi, O.** (2007) Molecular mechanisms of light-stress of photosynthesis. *Ann. N.Y/ Acad. Sci.* 1113:114-122. **IF: 1.381**

4. Kós, P.B., Deák, Z., **Cheregi, O.**, Vass, I. (2008) Differential regulation of psbA and psbD gene expression, and the role of the different D1 protein copies in the cyanobacterium *Thermosynechococcus elongatus* BP-1. *Biochim. Biophys. Acta* 1777(1):74-78. **IF:4.302**

5. Sicora, C., Brown, C.M., **Cheregi, O.**, Vass, I. and Campbell, D.A. (2008) The *psbA* gene family responds differentially to light and UVB stress in *Gloeobacter violaceus* PCC 7421, a deeply divergent cyanobacterium. *Biochim. Biophys. Acta* 1777(2):130-9. **IF: 4.302**

6. * Zsolt Balogi, **Otilia Cheregi**, Kim C. Giese, Kata Juhász, Elizabeth Vierling, Imre Vass, László Vígh and Ibolya Horváth (2008) A mutant small Heat Shock Protein with increased thylakoid association provides an elevated resistance against UV-B damage in *Synechocystis* 6803. Submitted to *J. Biol. Chem.*