Abstract of PhD Thesis

Molecular Mechanism of UV-B Damage and Repair of the Photosynthetic Apparatus in the Cyanobacterium Synechocystis sp. PCC 6803

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Introduction

Photosynthetically relevant solar radiation that reaches the Earth is divided into three main spectral regions: UV-B (280-320 nm), UV-A (320-400nm) and photosynthetically active radiation, PAR (400-700 nm). Among those, the UV-B region is selectively attenuated by the stratospheric ozone layer. On the contrary, UV-A and PAR radiation have no selective absorber and are affected mainly by light scattering. The biologically most damaging wavelengths below 280 nm, such as the UV-C (200-280 nm) region, are absorbed almost completely by the atmosphere and, therefore, are insignificant for biological processes under natural conditions.

Recent reduction in the stratospheric ozone layer as a consequence of human activities, allowing more UV-B radiation to reach the Earth surface and into the ecologically significant depths (10-15 m) of the ocean, is of concern because of the potential adverse effects on biosphere. An important action site of UV-B radiation in plant cells is the photosynthetic apparatus, in which a sensitive target is the light energy-converting complex of PSII. *In vitro* studies indicate that the most UV-sensitive component of PS II electron transport is the water-oxidizing complex. The Q_A and Q_B quinone electron acceptors as well as the Tyr-Z and Tyr-D redox-active tyrosine donors are also potential targets of UV-B, but their damage appears to be slower than the impairment of water oxidation.

An important consequence of UV-B radiation is the degradation of the D1 and D2 subunits of the PS II reaction center. Despite intense research, the molecular mechanism of II UV-B-induced inhibition and recovery of PSII still remains unclear. Yet it was shown that under low levels of UV-B radiation there is an enhanced turnover of both D1 and D2 proteins of PS II complex in higher plants. The so-called D1 protein repair cycle is known to facilitate the restoration of PS II function after photoinhibition, caused by visible light, which damages mainly the D1 protein. This protein subunit of the membrane-bound PS II complex has a much higher turnover rate than the other PS II proteins. Thus, the D1 protein has to be replaced while the other PS II components are not newly synthesized. D1 protein subunit of PSII is coded by *psb*A gene in higher plants. In cyanobacteria a *psb*A multigene family code for the D1 protein subunit of the PS II reaction center. In *Synechocystis* 6803 there are three different *psb*A genes named: *psb*A1, *psb*A2, *psb*A3. The *psb*A1 is not transcribed under natural conditions and its function is unknown. The *psb*A2 and *psb*A3 genes are encoding a D1 protein which have the same aminoacid sequence. In *Svnechococcus* sp. PCC 7942 there are also three genes: *psb*A1, *psb*A2 and *psb*A3 encoding two different forms of the D1

protein (D1:1 and D1:2). Under basal conditions the D1:1 form is used. Under stress conditions, like high light or UV-B, the D1:1 form is exchanged with the D1:2 form coded by the *psb*A2 and *psb*A3 genes. If the stress is removed the D1:2 is rapidly changed to D1:1 form encoded by *psb*A1 gene. The interchange process in *Svnechococcus* is a complex process regulated at both transcriptional and translational levels.

In cyanobacteria, the photosynthetic system is tightly connected to the other principal metabolic pathways and is in itself a major metabolic sink for iron, nitrogen and carbon skeletons. The general assembly of the photosynthetic membranes in cyanobacteria is similar to that of higher plants; therefore, *Synechocystis* 6803 might serve as a powerful model for studying the molecular mechanisms of stress response and long-term adaptation. Being naturally competent, this cyanobacterium can easily be transformed and foreign DNA integrates into its genome at a high frequency. Recently, the complete nucleotide sequence of the *Synechocystis* 6803 genome has been determined and the collection of annotated data (CyanoBase) is available over the Internet. Moreover, a useful promoter probing vector has been constructed and the recently published 'plasmid shuffling' method allows the manipulation and mutational analysis of essential genes in the organism.

Aim of Study

- Investigation of the UV-B damaging effect on PSII and the recovery of the activity under visible light conditions.
- Investigation of the importance of temperature on the processes of UV-B damage and repair of PSII.
- Elucidation of the relationship between visible light and UV-B light in damaging the PSII activity.
- Determination of the importance of UV-B induced DNA-damage, in relation to the damage at the photosynthetic electron transport level.
- Comparison of the two different cyanobacterian response strategies to UV-B damage from Synechocystis sp and *Svnechococcus* sp.

Materials and Methods

Experimental models. Tylakoid membranes were isolated from spinach as described by Turcsanyi and Vass 2000 and were stored at -80^0C until use in 0.4M glucose, 5mM $MgCl_2$, 10mM NaCl and 40 mM Hepes (pH 7.5) at 2-3 mg/ml.

The *Synechosystis sp*. PCC 6803 cyanobacterial strain was cultured in BG-11 medium on shaker at 30^0C, 57$\mu Em^{-2}s^{-1}$ and in the presence of 5% CO_2.

The *Synechococcus sp*. PCC 7942 cyanobacterial strain was cultured in BG-11 medium on shaker at 30^0C, 57$\mu Em^{-2}s^{-1}$ and in the presence of 5% CO_2

Light treatment. UV-B was provided by a Vilbert-Lourmat VL-215M lamp, in combination with a 0.1 mm cellulose acetate filter yielding 6 $\mu Em^{-2}s^{-1}$ intensity at the surface of the samples. Visible light was produced by an array of halogen lamps in the 130-1300 $\mu Em^{-2}s^{-1}$ intensity range. Chlorophyll concentration of the treated samples was in the range of 6.5-10 μgChl/ml.

Fluorescence relaxation kinetics. Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured by a double-modulation fluorometer (P.S.Instrumens, Brno,CZ) (Trtilek et al. 1997) in the 150μs to 100s range as described in (Vass et al. 1999).

Oxygen evolution measurements. Steady-state rates of oxygen evolution were measured by using a Hansatech DW2 O_2 electrode at a light intensity of 1000 $\mu Em^{-2}s^{-1}$ in the presence of 0.5 mM 2,5-dimetyl-p-benoquinone as electron acceptor. Typically, 2 ml of cells were used in each measurement.

Thermoluminescence measurements. TL was measured by using a home-made device. 400 μl samples containing 50 μgChl were excited using flashes or continuous light at different temperatures. Light emission was measured while heating the sample from -40^0C to 70^0C.

Results and Discussion

1. UV-B induced damage and recovery of the photosynthetic activity in Synechocystis sp. PCC 6803.

In intact Synechocystis cells UV-B induces a decrease in oxygen evolution as a consequence of decrease in the photosynthetic activity. Under our experimental conditions a decrease of about 50 % of the initial oxygen evolution rate was obtained in about 120 minutes, with a high initial loss to about 60 % in the first 30 minutes. The *Synechocystis* cells have the capacity of recovery after UV-B damage if exposed to visible light. The rate of recovery is directly dependent on light intensity if this does not exceed the photoinhibition values. The rate of recovery is also dependent on the extent of the damage. Under normal light conditions (45 $\mu Em^{-2}s^{-1}$) from a damage value of 50 % of initial oxygen evolution activity, the recovery is complete in about two hours.

From our study it is evident that temperature is also one factor which influences the extent of the damage caused by UV-B and the rate of recovery. After an initial damage of 33 % of oxygen evolution activity the recovery at 20^0C goes very slow by reaching a value of about 45% in 150 min. If we shift the temperature of the sample to 30^0C, maintaining all the other parameters, the cells start to recover much faster reaching the 70% value in 30 min

2. Influence of temperature on the process of UV-B damage and repair of photosynthetic apparatus.

From our study it is evident that temperature is also one factor which influences the extent of the damage caused by UV-B and the rate of recovery. To prove that we used a chemical agent, benzyl-alcohol, that is known to affect the fluidity of cell membranes in order to induce physical changes that will be similar by those of temperature. During these experiments we observed that BA has an inhibitory effect on the function of the electron transport chain that may be caused by the inhibition of de novo protein synthesis.

3. UV-B and visible light interaction in damaging the PSII activity.

In our experiments we used three experimental models: intact *Synechocystis* cells, *Synechocystis* cells treated with lincomycin, and tylakoid membranes isolated from spinach. The experimental protocols used were treatment with visible light of different intensities, UV-B light, and the combination of the two. When there is no repair ,in lincomycin treated cells and isolated thylakoids, the inhibition of oxygen evolution by visible and UV-B light show an additive feature when applied together. This suggests that UV-B light and visible light damages the photosynthetic apparatus independently. When the repair mechanisms are intact this effect is shadowed by the fact that visible light is triggering the protein synthesis dependent repair mechanisms. This, at low intensities of visible light, can fully compensate the UV-B induced damage. However, at increasingly higher intensities the protective feature of visible light is obscured by its damaging effect until repair rate becomes insignificant.

Another interesting, and very important feature is the presence in UV-B treated samples of a fast phase in flash-induced fluorescence decay curves when measured in the presence of DCMU. This indicates the accumulation of PSII centers in which the Mn cluster is inactivated. The fast phase is absent in samples which were exposed simultaneously to UV-B and visible light, and could be explained by the fact that those centers which have the Mn-cluster damaged by UV-B are more sensible and rapidly inactivated by visible light. Also the fast phase is not present in samples treated with visible light alone suggesting the acceptor side photoinhibition as cause of damage.

4. Role of DNA repair.

UV-B is an important damaging factor for DNA. It induces mutations through pyrimidine dimer formation as well as other structural and biochemical modifications of the DNA molecule. The UV induced DNA damage is repaired by the activity of an enzyme called photolyase. In *Synechocystis sp.* PCC 6803 the photolyase enzyme is coded by slr0854 gene and we used in our experiments a mutant which had the gene inactivated and consequently lacks the light induced repair of DNA damage. In contrast to WT cells this mutant is not able to grow when visible light is supplemented by UV-B due to accumulation of DNA damage. However on a short time scale (up to a few hours) there is no significant difference on the rate of damage induced by UV-B and visible at the PSII level, between Δslr0854 and WT strains.

5. The difference between the two D1 forms from Svnechococcus regarding the UV-B sensitivity.

In cyanobacteria there are two different response strategies to UV-B irradiation: *Synechocystis sp*. PCC 6803 has three different *psb*A genes from which one (*psb*A1) is not expressed and the other two are coding the same D1 protein. In *Svnechococcus* sp. PCC7942 exists also three different psbA genes coding two types of D1 proteins (D1:1 and D1:2). Under UV-B light conditions *Svnechococcus* is exchanging D1:1 with D1:2. In order to see the difference between the two forms of D1 in regard to UV-B sensitivity we used mutants of *Synechocystis* which contain the gene for D1:1 and D1:2 respectively from *Svnechococcus*. We have shown that the mutant with D1:1 was more sensible than the mutant with D1:2. However both the mutants containing D1:1 and D1:2 showed a higher rate of damage and a lower capacity of recovery than WT.

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List of Publications

1. **Sicora, C.** and Vass, I. (2000) "The interaction of visible and UV-B light in damaging the electron transport of Photosystem II in the cyanobacterium *Synechocystis* 6803" Plant Physiol. Biochem. 38 (suppl.): 110
2. **Cosmin Ionel Sicora,** Mihail Dragan-Bularda and Imre Vass (2000) UV-B-Induced Damage and Recovery of Photosynthetic Activity in the Cyanobacterium

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3. I Vass, E Turcsányi, L Sass, A Szilárd, **C Sicora**, Z Máté, É Hideg, F. Nagy, A Viczián (2001) Damage and repair of Photosystem II under exposure to UV radiation. Proc. 12th Int. Congress on Photosynthesis, Brisbane, Australia

4. **Cosmin Sicora** and Imre Vass (2001) The Interaction of Visible and UV-B Light in Damaging the Electron Transport of Photosystem II in the Cyanobacterium *Synechocystis 6803***.** Satellite Meeting of the 12th International Congress on Photosynthesis, Aug 13-17, Heron Island, Australia (2001) pp. 15**4.**

5. **Cosmin Sicora,** András Szilárd, László Sass, Enikő Turcsányi, Zoltán Máté and Imre Vass (2003) UV-B and UV-A radiation effects on photosynthesis at the molecular level. NATO ASI Series Environmental UV radiation: Impact on Ecosystems and Human Health and Predictive Models. (Book chapter-in press)

6. **Cosmin Sicora,** Zoltan Mate and Imre Vass (2003) The interaction of visible and UV-B light during photodamage and repair of photosystem II. Photosynthesis Research 75: 127-137.

7. M. Tichy, L. Lupinkova, **C. Sicora**, I. Vass, O. Prasil and Josef Komenda (2003) *Synechocystis* 6803 mutants expressing distinct forms of the photosystem II D1 protein from *Synechococcus* PCC 7942: Relationship between the D1 sequence and sensitivity to visible and UV-B radiation. Submitted to Biochem, Biophys. Acta.