Thesis Summary

STUDIES ON THE MECHANISM OF PHOTODAMAGE AND PHOTOPROTECTION OF PHOTOSYSTEM II IN THE CYANOBACTERIUM *SYNECHOCYSTIS* PCC 6803

Submitted by

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Introduction:

Photosynthesis is a process in which green plants, algae, cyanobacteria utilize energy from sunlight to manufacture carbohydrates from CO_2 and water. It is the main source of energy for all the plants to drive their metabolic process. The photosynthetic apparatus contain four protein complexes in the thylakoid membrane namely Photosystem I (PSI), Photosystem II (PSII), cytochrome b_6/f and ATP synthase (Renger 2010). These photosystems are composed of reaction centers where charge separation occurs. There are several studies describing the high light stress on PSII (Ruban et al. 2007; Abasova et al. 2011; Vass 2012). Excess light reaching the photosynthetic apparatus causes photodamage and decline of photosynthetic activity which is known as photoinhibition (Aro et al. 1993; Vass and Aro 2008). It leads to the inactivation of PSII and results in the damage to the reaction center, mainly the D1 protein subunit. This damage is followed by the repaired D1 protein (Ohad et al. 1984; Prasil et al. 1992). In living organisms, photoinhibited PSII centers are continuously repaired via degradation and synthesis of the D1 protein of the photosynthetic reaction center of PSII. Light stress to PSII becomes a problem when the rate of photodamage exceeds the capacity of repair process. The photodamage can be monitored in the presence of protein synthesis inhibitors such as lincomycin or chloramphenicol (CAP), which blocks the repair. Therefore the rate of photodamage can be monitored separately from the rate of repair. Reactive oxygen species (ROS) are produced when the cells are subjected to stress conditions. ROS includes singlet excited oxygen, superoxide and hydroxyl radicals and peroxides. It has been reported that superoxides are produced by photosynthetic organisms by photoreduction of oxygen by electron transport from acceptor side of PSI (Asada 2006). It has also been reported previously that PSII is involved in the generation of superoxide and the Plastoquinone pool has also been involved for the generation of superoxide (Pospíšil 2009). CAP has been reported earlier that it serves as an electron acceptor as it takes electron from PSI to molecular oxygen and competes with CO_2 reduction (Okada et al. 1991). Despite the fact that CAP is a known electron acceptor, subsequent groups continued to apply it as a protein synthesis inhibitor in photoinbition studies. Therefore the action and the nature of CAP remained controversial.(Nishiyama et al. 2001, 2005; Allakhverdiev et al. 2005; Takahashi and Murata 2008; Murata et al. 2012).

The afterglow (AG) thermoluminescence band, peaking around 45 ºC, originates from heat induced back transfer of electrons from stroma to the acceptor side of PS II to Q_B in $S_{2/3}Q_B$ centers which creates the $S_{2/3}Q_B^-$ state (Sundblad et al. 1990; Miranda and Ducruet

1995; Havaux 1996; Ducruet et al. 2005). The AG band is related to CEF and transmembrane proton gradient formation in the thylakoids, it is a useful diagnostic tool to study photosynthetic activity under various conditions as discussed in detail in a recent review article (Ortega and Roncel 2021). We tested specific experimental conditions, which were expected to induce temperature-dependent reduction of the PQ pool via CEF in *Synechocystis* 6803.

3.0. Aims of the study:

The general aim of the project was to investigate the mechanism of photosynthetic electron transfer in isolated thylakoid membrane complexes, as well as cyanobacteria in order to reach better understanding the mechanism of photoinhibition of PSII and cyclic electron flow.

The specific aims of this study were:

1, To understand the mechanisms of photodamage of the PSII complex in the presence of chloramphenicol in isolated thylakoid membrane particles.

2, To understand the mechanisms of photodamage of the PSII complex in the presence of chloramphenicol in intact *Synechocystis* cells.

3, To obtain a better understanding of CEF in cyanobacteria, by utilizing the potential of the AG afterglow thermoluminescence phenomenon

3. Materials and methods

Wild-type *Synechocystis* cells were grown autotrophically and photomixotrophically in BG-11 growth medium in a rotary shaker at 30 °C under a 3% CO_2 enriched atmosphere. The intensity of light during growth was 40 μmol photons m^{-2} s^{-1}. PSI-less cells were grown at low light intensity (of 5 μmol photons m^{-2} s^{-1}) in the presence of 5 mM glucose and 25 μg mL^{-1} spectinomycin. As a control for the PSI-less strain, WT cells were also cultured under same conditions.

Cells were harvested by centrifugation at 8000 g for 10 min and resuspended in 100 mL fresh BG-11 medium at 5 μg Chl mL^{-1} concentration. Before starting high light treatment, cells were incubated for 1 h under growth light (40 μmol photons m^{-2} s^{-1}) followed by measuring the control value of oxygen evolution, which was used as zero time point for the high light treatment. For photoinhibitory treatment, cells were illuminated with 500 μmol photons m^{-2} s^{-1} white light without additions, in the presence of the protein synthesis inhibitor lincomycin (300, or 400 μg mL^{-1}) or chloramphenicol (50, 100, 200 μg mL^{-1}). Steady-state O_2 evolution rates were measured by using a Hansatech DW_2 O_2 electrode at 30 °C under illumination with 2300 μmol m^{-2} s^{-1} light intensity in the presence of 0.5 mM DMBQ as an

artificial electron acceptor. Statistical analysis was performed by one way ANOVA using the Tukey test for means comparison. The calculations were done by the Origin 2018 graphics software.

Thylakoid membranes were isolated from fresh spinach leaves as described earlier by (Berthold et al., 1981), and suspended in a buffer solution containing 50 mM tricine (p^H 7.5), 7 mM $MgCl_2$, 7 mM $CaCl_2$ and 0.3 M Sorbitol. Photosystem II (PSII) membrane particles were isolated from fresh spinach leaves as described earlier by (Berthold et al. 1981; Vass et al. 1987), and suspended in a buffer solution containing 40 mM MES-NaOH (pH 6.5), 15 mM $MgCl_2$, 15mM $CaCl_2$ and 1M Betaine.

The resuspended thylakoid membranes of 40 mL volume at a concentration of 25 µgchl/mL were illuminated at the irradiation of 500 µmole m^{-2} s^{-1} with and without chloramphenicol at (200 µg/ml). The temperature during illumination was maintained at 4 °C. The resuspended thylakoid membranes were also illuminated at the irradiation of 500 µmole m^{-2} s^{-1} with chloramphenicol at (200 µg/ml) and superoxide dismutase (20 units/mg). Oxygen evolution of irradiated thylakoid membranes was measured in 1 mL aliquot using a DW_2 oxygen electrode (Hansatech) at 4 °C under the illumination of 2300 µmole $m^{-2}s^{-1}$ visible light intensity in the presence of artificial electron acceptors 0.5 mM DMBQ at different illumination time points (0, 15, 30, 45) .The PSII particles were resuspended at 5 µg Chl mL^{-1} in 40 mL volume and illuminated with 500 µmole m^{-2} s^{-1} light intensity in the presence and absence of CAP (200 µg/mL). The temperature during illumination was maintained at 4 °C. The samples were also illuminated in the presence of SOD (20 units mg^{-1}). For monitoring PSII activity the rate of O_2 evolution was measured at the indicated time points. Photosynthetic activity of irradiated PSII membranes was also assessed by measuring the so called OJIP transient of variable Chl fluorescence during application of a 2s saturating pulse (Strasser et al., 1995) by using an FL-3000 fluorometer (PSI). F_v/F_m was obtained by calculating $(F_m-F_o)/F_m$, where F_o and F_m represent the minimum fluorescence in dark adapted sample, and the maximal fluorescence yield under continuous saturating light, respectively.

Flash induced thermoluminescence (TL) measurements were performed as described in (Cser and Vass 2007). Cells equivalent to 30 µg of Chl were pre-illuminated for 30 s with white light at room temperature and then dark adapted for 3 min at room temperature. Samples without addition were illuminated with a single turnover saturating flash given at +10 ºC with or without an additional far-red (FR: 740 nm) illumination, provided by an LED source during cooling of the samples from 0 to -40 ºC, or from -10 to -40 ºC . To block the

electron transport at the Q_B site of PSII, 10 μM DCMU was added in the dark and incubated for 3 min followed by FR illumination from -10 º to -40 ºC. After cooling the samples to -40 ºC they were heated to 80 ºC at a rate of 20 ºC min^{-1} and the luminescence intensity was recorded as a function of temperature.

Chlorophyll fluorescence was measured with the Dual-PAM-100 chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) at 30 ºC. A slow induction measuring protocol was launched and the Chl fluorescence signal was recorded for 30 s in dark to establish a baseline, then the actinic red light (635 nm peak intensity) at ~56 μmol photons $m^{-2}s^{-1}$ was turned on to record light-induced Chl fluorescence variation during 180 s, and thereafter the fluorescent signal was recorded during the light-to-dark transition for an additional 75 s, i.e. to a maximum of 255 s.

Results

- Our data show that CAP can accept electrons at the acceptor side of Photosystem II, most likely from Pheophytin, and deliver them to molecular oxygen leading to superoxide production. In addition, the presence of CAP enhances photodamage of Photosystem II electron transport in isolated BBY particles, which effect is reversible by superoxide dismutase (Rehman et al. 2016). We show that CAP acts as an electron acceptor in Photosystem II and mediates its superoxide dependent photodamage. Besides blocking the repair cycle of PSII, CAP might accelerate the rate of photodamage leading to artifacts concerning the mechanism of photoinhibition.
- Our results show that the rate of PSII photodamage was significantly enhanced by CAP, at the usually applied 200 μg mL^{-1} concentration, relative to that obtained in the presence of lincomycin. The chloramphenicol-induced enhancement of photodamage was observed not only in wild-type *Synechocystis* 6803, which contains both Photosystem I (PSI) and PSII, but also in a PSI-less mutant which contains only PSII. Importantly, the rate of PSII photodamage was also enhanced by the absence of PSI when compared to that in the wild-type strain under all conditions studied here, i.e., without addition and in the presence of protein synthesis inhibitors. We conclude that chloramphenicol enhances photodamage mostly by its interaction with PSII, leading probably to superoxide production. The presence of PSI is also an important regulatory factor of PSII photodamage most likely via decreasing excitation pressure on PSII (Kodru et al. 2020).

- The so-called afterglow, AG, thermoluminescence (TL) band is a useful indicator of the presence of cyclic electron flow (CEF), which is mediated by the NADH dehydrogenase-like (NDH) complex in higher plants. Although NDH-dependent CEF occurs also in cyanobacteria, the AG band has previously not been found in these organisms. We tested various experimental conditions and could identify a TL component with ca. +40 ºC peak temperature in *Synechocystis* PCC 6803 cells, which were illuminated by far-red (FR) light at around -10 ºC. The +40 ºC band could be observed when WT cells were grown under ambient air level CO_2, but was absent in the M55 mutant, which is deficient in the NDH-1 complex. These experimental observations match the characteristics of the AG band of higher plants. Therefore, we conclude that the newly identified +40 ºC TL component in *Synechocystis* PCC 6803 is the cyanobacterial counterpart of the plant AG band and originates from NDH-1-mediated CEF. The cyanobacterial AG band was most efficiently induced when FR illumination was applied at -10 ºC and its contribution to the total TL intensity declined when cells were illuminated above and below this temperature. Based on this phenomenon we also conclude that CEF is blocked by low temperatures at two different sites in *Synechocystis* PCC 6803: (1) Below -10 ºC at the level of NDH-1 and (2) below -30 ºC at the donor or acceptor side of Photosystem I (Kodru et al. 2021).

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Publications related to the PhD thesis:

- Kodru S, Rehman AU, Vass I (2020) Chloramphenicol enhances Photosystem II photodamage in intact cells of the cyanobacterium *Synechocystis* PCC 6803. Photosynth Res 145:227–235 (IF: 3.2).
- Rehman AU, Kodru S, Vass I (2016) Chloramphenicol mediates superoxide production in photosystem II and enhances its photodamage in isolated membrane particles. Front Plant Sci 7:1–5. (IF: 4.9)
- Kodru S, Sass L, Patil P, et al (2021) Identification of the AG afterglow thermoluminescence band in the cyanobacterium *Synechocystis* PCC 6803. Physiol Plant 171:291–300. (IF: 4.1)

Other publications:

- Patil PP, Vass I, Kodru S, Szabó M (2020) A multi-parametric screening platform for photosynthetic trait characterization of microalgae and cyanobacteria under inorganic carbon limitation. PLoS ONE 15(7): e0236188. (IF: 2.7)

International conference abstracts and poster presentations

- Sandeesha Kodru, Ateeq ur Rehman and Imre Vass: How does chloramphenicol affect PSII photoinhibition in isolated spinach thylakoid membranes? (Straub conference at BRC Szeged, Hungary, 3-4 June,2015).
- Sandeesha Kodru, Ateeq ur Rehman, Milán Szabó and Imre Vass: The role of chloramphenicol in enhancing photodamage and oxygen uptake in isolated PSII, thylakoids and in intact *Synechocystis* (Straub conference at BRC Szeged, Hungary, 24-25 may,2018)
- Sandeesha Kodru, Ateeq Ur Rehman, Imre Vass: Chloramphenicol enhances Photosystem II photodamage via superoxide Production (14th Nordic Photosynthesis Congress,Turku, Finland 22-24 May 2019)
- Imre Vass, Sandeesha Kodru, Ateeq Ur Rehman: Singlet oxygen production and photodamage of Photosystem II are enhanced in the absence of Photosystem I in *Synechocystis* PCC 6803 (14th Nordic Photosynthesis Congress, Turku, Finland 22-24 May 2019)
- Sandeesha Kodru, Ateeq Ur Rehman, Imre Vass: Chloramphenicol enhances Photosystem II photodamage via superoxide Production (Straub conference at BRC Szeged, Hungary, 30-31 may, 2019)

Declaration

As the corresponding author, I declare that the author Sandeesha Kodru contributed significantly to the results of the scientific publications listed below. I attest that the results presented in this thesis were not presented in any other PhD thesis.

i. **Kodru S**, Rehman AU, Vass I (2020) Chloramphenicol enhances Photosystem II photodamage in intact cells of the cyanobacterium *Synechocystis* PCC 6803. Photosynth Res 145:227–235.

ii. Rehman AU, **Kodru S**, Vass I (2016) Chloramphenicol mediates superoxide production in photosystem II and enhances its photodamage in isolated membrane particles. Front Plant Sci 7:1–5.

iii. **Kodru S**, Sass L, Patil P, et al (2021) Identification of the AG afterglow thermoluminescence band in the cyanobacterium *Synechocystis* PCC 6803. Physiol Plant 171:291–300.

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