Thesis of the PhD dissertation

The PhrA photolyase and its homologue, the PhrB cryptochrome play important roles in the efficient PSII repair cycle of *Synechocystis* sp. PCC6803

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Szeged

2014

Introduction

Sunlight, besides being the energy source of the biosphere, is also its regulation factor. Photoautotrophic organisms are unavoidably exposed to the Sun's radiation while harvesting its energy via pigment-protein complexes (PSII, PSI, cyt b6/f, ATP synthetase) and being regulated via specific light receptors (phytochromes, cryptochromes, phototropins, UVR8). Both of these processes require that the respective molecules and molecule complexes endure temporary structural and/or redox modifications.

Parallel to these phenomena, depending on the wavelength and intensity of the radiation, the aforementioned molecules may suffer permanent damages as well. This damaging effect is not necessarily restricted to molecules that have a functional role in being exposed to radiations, it is rather a general phenomenon, affecting every single molecule, which is capable to absorb in the respctive wavelength range. The most important among these is DNA, which absorbs heavily in the UV range and UV-B (280-315 nm) specifically produces structural changes in the molecule.

Under normal circumstances the molecules modified via radiation from the Sun are repaired or replaced to ensure their functionality. These repair processes are of utmost importance as the efficiency and continuity of photosynthesis depends on them.

My thesis discusses the role of two proteins in the repair of photosystem II (PSII) of the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*).

Within the photosynthetic apparatus the repair of PSII enjoys high priority, mostly because its D1 subunit holds or binds most of the redox factors, required for the initial charge separation and electron transfer in the light phase of photosynthesis. In addition to this D1 also provides several binding moieties for the oxygen evolving complex.

Because of their role in processing and transferring radiational energy, the subunits of PSII D1 and D2 suffer regular damaging and are replaced at a relatively high rate. Photosynthetic organisms invest a great amount of energy in keeping these repair and replacement processes swift and efficient.

The most imoprtant steps of PSII repair are the replacement of the D1and D2 subunits. The repair process itself consists of several steps: (1) Proteolytic removal of the damaged D1 and D2 protein subunits, (2) Transcription of mRNA from the *psbA* and *psbD* genes encoding the D1 and D2 subunits, respectively, (3) Production of new protein subunits from the respective mRNA pools, (4) Incorporation of the newly synthesized protein copies into the PSII complex, and finally (5) Religation of redox cofactors and reactivation of PSII. If this process becomes ineffective, photoinhibition will take over and photosynthesis will slow down and eventually stop.

Besides the photosynthetic apparatus DNA is another major target of UV-B. The energy of UV-B radiation forms dimers, mostly cyclobutane pyrimidine dimers (CPDs), in the DNS's structure, which obstruct the function of RNA and DNA polymerases. This effect, depending on how fast the organism is able to rid itself from the dimers, may quickly turn lethal. Photolyase (PhrA) is an enzyme, which, using the energy of UV-A radiation and/or blue light is able to specifically catalyse the monomerization of CPDs, in a way that is quicker and more energy efficient than other, non specific processes that rely on the excision of the damaged sites. The process catalysed by photolyase is termed photoreactivation.

PhrB is the structural homologue of the *Synechocystis* photolyase (PhrA) enzyme. It has been a decade since PhrB has been identified and, based on its amino acid sequence, assigned to a group of molecules called DASH cryptochromes (*Drosophila, Arabidopsis, Synechocystis, Homo*). However, despite the structural similarities, the exact function or rather functions of PhrB have not been clearly established to this day. It has been proposed that it acts as a weak photolyase, a transcriptional repressor and a blue light receptor as well. All these data point towards a protein with diverse functions.

Goals

Light stress significantly enhances the rate of protein replacement in the photosynthetic apparatus. One of the crucial steps of the PSII repair cycle is the replacement of the D1 protein subunit, which requires the upregulated synthesis of *psbA* mRNAs. Because UV-B radiation potentially hinders the synthesis of *psbA* mRNAs, the question arises whether UV-B induced DNA damage has any direct or indirect effect on the recovery process of PSII?

To answer this we studied the following:

1. What effect, if any, has the lack of the DNA repair photolyase (PhrA) enzyme on the PSII repair cycle in the cyanobacterium *Synechocystis* sp. PCC 6803?

2. What role does PhrB, homologue protein of PhrA, play in the UV-B induced DNA repair and in the PSII repair cycle of the cyanobacterium *Synechocystis* sp. PCC 6803?

Materials and methods

Culturing *Synechocystis* wild type and mutant cell lines.

UV-B and high intensity photosynthetically active radiational (PAR) stresses.

PSII activity estimation via flash induced chlorophyll-a fluorescence and oxygen production.

Thylakoid membrane isolation, gel electrophoresis, immunoblotting.

Native 2D electrophoresis.

Total RNA isolation, reverse transcription, qPCR.

Genomic DNA isolation, alkaline gel electrophoresis.

Results and discussion

In order for us to study the effect of DNA photoreactivation on PSII recovery we used a Synechocystis mutant which didn't have a functional photolyase enzyme (*phrA*$^-$), hence its specific DNA repair process of UV-B-induced damages was greatly impaired. Comparing *phrA*$^-$ *cells* to the wild type (WT) *Synechocystis* we found that under UV-B radiation the mutant cells lost a greater portion of their PSII activity and at a higher rate as well. The addition of protein synthesis inhibitor lincomycin to the cell lines made it clear that the extreme UV-B sensitivity of the *phrA*$^-$ cells originated in the *de novo* protein synthesis step of the PSII repair cycle.

Since it is the protein with the fastest replacement rate within the thylakoid membrane, we concentrated on the *de novo* synthesis of D1. Isolating thylakoid membranes from cells under and after UV-B stress we found that the D1 pool did not

change significantly in the WT. In contrast the *phrA*$^{-}$ cells lost a significant portion of their D1 pool, which proved to be permanent, as they were unable to recover it even in the absence of UV-B radiation.

In theory efficient *de novo* protein synthesis relies on an available and abundant mRNA pool. To test whether *phrA*$^{-}$ cells had enough *psbA3* at their disposal, which is the stress-induced isoform of the *psbA* gene family coding for D1, we carried out transcript analysis on both cell lines. In concert with the previous data *psbA3* in WT showed a considerable induction upon exposure to UV-B and an expected decrease when the stress factor was eliminated. A repeated UV-B exposure proved that the psbA3 stayed just as inducable in a second stress phase, as it was initially, thus the genome probably did not suffer permanent damage. Unlike in the WT, at the end of the UV-B treatment, the *psbA3* induction dropped significantly in *phrA*$^{-}$ cells, compared to their initial rise, and mirroring the permanent effect on the D1 pool, this did not change even in the absence of UV-B radiation. Induction of *psbA3* in the *phrA*$^{-}$ cells was also absent in the second UV-B treatment, pointing towards a greatly damaged genome. This assumption was underlined by the fact that besides the absence of the stress gene induction, the mutant cells were unable to degrade the already existing mRNA pool, giving rise to the hypothesis that UV-B radiational damage was not limited to the *psbA* genes, rather it affected the whole genome.

To quantify the UV-B-induced DNA damage, we applied two methods: a qPCR method and an alkaline gel electrophoresis method. The qPCR method does not distinguish between DNA damages as it takes every DNA polymerase blocking damage into account. Applying this we found that at the end of the UV-B treatments, in average every 1 kb DNA from *phrA*$^{-}$ cells contained close to 1 DNA polymerase blocking damage. Since the average gene length of Synechocytsis is 1 kb, this is a significant amount of damages. In order to determine how much of these damages are CPDs, specifically induced by UV-B we digested genomic DNA (gDNA), isolated from UV-B treated WT and *phrA*$^{-}$ cells, with the T4 Endonuclease V PDG enzyme. This enzyme specifically cleaves DNA strands at CPD sites, hence the more substrate it finds on the molecule, the shorter fragments will result from the digestion. Separated on an alkaline gel, short fragments will form a smudge, and the farther this smudge runs on the gel, the shorter the fragments are, hence the more CPDs were on the DNA. Analysing the gel image we were able to approximate the amount of CPDs

which significantly overlapped with our qPCR data, proving that most DNA polymerase blocking damages on the genome of *phrA*$^-$ cells, causing the higher UV-B sensitivity, were in fact CPDs.

We studied the effect of PhrB by comparing WT and *phrB*$^-$ cells. As a result we found that *phrB*$^-$ cells lost a bigger portion of their PSII activity not only to UV-B, but also to high intensity PAR stress. This let us assume that the effect of PhrB is not restricted to UV-B alone rather it is a general event. With the help of lincomycin we once again arrived at the conclusion that, similar to *phrA*$^-$ cells, the enhanced UV-B and PAR sensitivity of *phrB*$^-$ cells is due to an inefficient de novo synthesis during PSII repair.

However, it quickly turned out that in the absence of PhrB, unlike in the case of the missing photolyase, CPDs did not accumulate in the gDNA nor did detectable amounts of one of the most frequently occuring oxidates damages, 7,8-dihidro-8-oxoguanin. Thus we found that despite the structural similarities PhrB, unlike PhrA, does not exert its effect on the PSII repair process through the specific DNA repair.

Nevertheless PhrB does have a significant effect on PSII repair as its absence greatly influences the D1 pool of *phrB*$^-$ cells, which ended up to be significantly lower than that of the WT cells, both under UV-B and PAR.

Since *phrB*$^-$ cells did not accumulate detectable amounts of DNA damages, it was of no surprise that their *psbA3* levels were similar to the WT's. This phenomenon indicated that PhrB acts either at the level of protein degradation, *psbA* transcription or PSII assembly. The effect on degradation could be excluded however, because the process occurs efficiently even in the absence of PhrB.

As the effect of PhrB firmly targeted protein synthesis, we intended to find out whether this includes other proteins as well, besides D1. Applying 2D native gel analysis, we found that in *phrB*$^-$ cells the amount of several proteins decreased. Among these the most notable ones were SbtA, a bicarbonate transporter, RbcS the small subunit os RuBisCO and PilA1, a motility protein of Synechocystis which is also assumed to be able to bind chlorophyll.

Both, the decrease of SbtA and the RsbC have the potential to lower the efficiency of *psbA* transcription because with their decrease the efficiency of the

Calvin-Benson cycle will drop as well, which results in the generation of reactive oxygen species, that specifically hinder D1 synthesis. The PilA1 most probably has an important role in the assembly of PSII complexes, through its potential ability to bind and transfer chlorophyll molecules.

Conclusions

- UV-B induced DNA damages affect photosynthesis in a direct manner.
- The lack of photolyase (PhrA) enhances UV-B induced PSII damages, hence, it increases UV-B sensitivity.
- The increased UV-B sensitivity results from the lack of specific DNA repair, that significantly lowers the efficiency of *psbA* transcription, which otherwise is indispensable for PSII repair.
- The two repair processes that alleviate UV-B-induced damages, the PSII repair cycle and the DNA repair cycle are directly connected to each other.
- The absence of PhrB lowers the ability of Synechocytsis cells to maintain an efficient PSII repair cycle under UV-B and high intensity PAR.
- PhrB, unlike the photolyase does not act as a specific DNA repair enzyme.
- PhrB affects several proteins, among which SbtA, RbcS and PilA1 can be associated either to the *psbA* transcription or to the PSII assembly.

Publications

The thesis was based on the following:

Vass IZ, Kós PB, Sass L, Nagy CI, Vass I. "The ability of cyanobacterial cells to restore UV-B radiation induced damage to Photosystem II is influenced by photolyase dependent DNA repair", Photochemistry and Photobiology (2013) 89: 384–390.

Vass IZ, Kós PB, Knoppová J, Komenda J, Vass I "The cry-DASH cryptochrome encoded by the *sll1629* gene in the cyanobacterium *Synechocystis* PCC 6803 is required for Photosystem II repair", Journal of Photochemistry and Photobiology (2014) 130: 318–326

Other publications:

Fodorpataki L, **Vass IZ** "Changes in chlorophyll fluorescence during the greening of etiolated leaves", Studia Universitas Babeş-Bolyai, Biologia (2005) issue no 1.

Vass IZ "The effect of environmental pollutants on the induced chlorophyll fluorescence of greening leaves", Acta Scientiarium Transylvanica – Múzeumi Füzetek (2007) 14-1.

Bírálat alatt: Nagy CI, Vass I, Rákhely G, **Vass IZ**, Tóth A, Duzs Á, Peca L, Kruk J, Kós PB "Functional link between sulfide:quinoneoxidoreductase and arsenic resistance genes in Synechocystis sp. PCC6803 regulated by an ArsR-type repressor of dual substrate specificity: a reminiscence of ancient anaerobic metabolism

Vass István Zoltán "Egy baktérium a Napra néz" Élet és Tudomány LXIX 10:294-296.