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UVB-induced DNA and photosystem II damage in two intertidal green macroalgae: Distinct survival strategies in UV-screening and non-screening Chlorophyta

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ABSTRACT

Ultraviolet-B-induced (UVB, 280–315 nm) accumulation of cyclobutane pyrimidine dimers (CPDs) and deactivation of photosystem II (PS II) was quantified in two intertidal green macroalgae, *Ulva clathrata* and *Rhizoclonium riparium*. The species were chosen due to their shared habitats but contrasting UVB screening potentials. In the non-screening *U. clathrata* CPDs accumulated and PS II activity declined as a linear function of applied UVB irradiance. In *R. riparium* UVB-induced damage was significantly lower than in *U. clathrata*, demonstrating an efficient UVB protection of DNA and PS II by screening. Based on the UVB irradiance reaching the chloroplasts, both species showed an identical intrinsic sensitivity of PS II towards UVB, but DNA lesions accumulated slower in *U. clathrata*. While repair of CPDs was similar in both species, *U. clathrata* was capable of restoring its PS II function decidedly faster than *R. riparium*. In *R. riparium* efficient screening may represent an adaptation to its high light habitat, whereas in *U. clathrata* high repair rates of PS II appear to be important to survive natural UVB exposure. The role of shading of the nucleus by the large chloroplasts in *U. clathrata* is discussed.

1. Introduction

Ultraviolet-B (UVB, 280–315 nm) radiation comprises only a small part of the solar spectrum but has multiple negative effects on the biosphere. Especially the potential to damage DNA can be critical for the growth and survival of an organism [57,10,26,33]. Different types of DNA lesions are induced by absorbing high energy quanta of the UVB range of the solar spectrum [19,15]. The most frequent ones are cyclobutane-pyrimidine dimers (CPDs) as measured in irradiated isolated DNA as well as in intact cells [22,3]. Other possible DNA lesions are the 6,4 photoproducts (6,4-PPs) which comprise about 25% of the total dimers [49,48]. Both types of lesions impede the progression of DNA and RNA polymerases and therefore limit DNA replication and transcription [69]. As a cytotoxic consequence, UVB-induced DNA damage leads to drastic restrictions in metabolism. Due to imprecise repair or damage bypass by polymerases, DNA damage accumulation can also exhibit mutagenicity that is clearly related to carcinogenesis [59]. Thus, the quantification of DNA damage is an important parameter for determining the UVB resistance of an organism.

In plants, another sensitive target of UVB radiation is the photosystem II (PS II) complex, especially the manganese cluster of the water splitting apparatus [55,75,77]. After absorption of UVB the manganese ions are released from the cluster and electron flow towards PS II reaction center is inhibited [27]. Furthermore, aromatic components of PS II like the donor to P680, tyrosine, Y_Z, or plastoquinones Q_A and Q_B on the acceptor side, may absorb UVB and be damaged, also resulting in impaired electron transport [76]. As a consequence $P680^+$ may accumulate and an oxidative chain reaction is activated degrading the associated proteins of PS II, e. g. the D1 reaction center protein [55]. Measuring the photosynthetic efficiency of PS II can therefore give valuable insights into the UV resistance or susceptibility of a plant species.

Resistance against UVB radiation can be achieved either by cellular tolerance mechanisms or by screening of UVB radiation. Cellular tolerance towards UVB radiation depends on the balance between the rates of damage induction and repair processes [43].

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With respect to CPDs a very important UV tolerance mechanism is the so called photoreactivation. During this process the enzyme photolyase is activated by UVA radiation and blue light and splits the covalent bond of a pyrimidine dimer (reviewed in [67,11]). In *Ulva pertusa* this mechanism is suggested to be very efficient in restoring spore germination [28]. In the dark, CPDs can be removed from the DNA strand by nucleotide excision repair (NER) or base excision repair (BER) [68]. However, photoreactivation is much faster than dark repair [56]. The rate of NER and BER depends on factors as type of lesion, methylation, transcription, condensation degree and DNA–histone interaction [9,29,17,40]. For photosynthesis, it was shown that the D1 protein has a higher turnover in UVB tolerant microalgae than in sensitive species [78].

The other type of resistance mechanism, UV screening by photoprotective pigments, is widespread among aquatic and terrestrial phototrophs [21,66,71]. UVB absorbing compounds have been shown to increase the UV resistance of *Arabidopsis thaliana* [44], rice [32], the dinoflagellate *Gymnodinium sanguineum* [53] or the Antarctic moss *Ceratodon purpureus* [20]. On the other hand, most green macroalgae are lacking UV protective compounds with only a few exceptions [38,34]. This is supported by an *in vivo* UV-screening study for a large number of marine green macroalgal species [58]. However, in this study it was also found that species of the order Cladophorales displayed considerable screening capacity for UVB and UVA radiation. So far, it has not been shown that this screening provides photoprotection.

Therefore, susceptibility of two different targets of UVB radiation, DNA and PS II, was investigated in a green macroalga of the order Cladophorales, *Rhizoclonium riparium*. For comparison the non-screening species *Ulva clathrata* (Ulvales) was used. Both species inhabit the upper eulittoral zone from temperate coasts [50]. There, emergence occurs regularly during low tide and the thalli are exposed to direct sunlight, including UVB radiation. For that reason, they are at high risk to accumulate DNA damages or reductions in photosynthetic efficiency [42] and have to employ UVB defense mechanisms. Exposed eulittoral species are reported to be very stress-resistant, especially towards high light stress [30,54] and UVB radiation [4,6,63]. It has been observed that at high photosynthetically active radiation (PAR) the relative contribution of UVB radiation to damage is decreased (reviewed in [23]). Therefore, we deliberately chose an artificial light regime lacking significant proportions of PAR. This approach enables assessing UVB induced damages to PS II separated from PAR driven confounding photoinhibitory effects.

We hypothesized that the UV-screening species *R. riparium* displays a significantly higher resistance than the non-screening species *U. clathrata*. The species-specific response is expected to be correlated to the observed difference in screening. Since both algae share the same habitat, we further hypothesized that *U. clathrata* employs alternative physiological resistance mechanisms to survive UV radiation (UVR) stress, e.g. efficient repair mechanisms.

Furthermore, we also want to highlight a methodological aspect of DNA damage measurements. UVB-induced CPDs have been detected routinely with a well-established dot blot immunoassay in various organisms ([14] (bacterio- and phytoplankton), [61] (macrophytes), [73] (maize)). This chemiluminescence detection assay relies on a calibrated CPD standard that allows obtaining absolute values and comparability between studies. Otherwise only relative data obtained within single blots are comparable (e.g., [74,17]). This limits the investigations severely with respect to sample size and reproducibility. As there was no calibrated CPD standard available, we made the effort to go back to the original protocol of Sinha et al. [72] and created a new standard. As this problem may be encountered also by others we describe in detail how this standard was prepared and how it was used to quantify CPDs.

2. Material and methods

2.1. Algal material

Unialgal cultures of clones from *U. clathrata* (Roth) C. Agardh (order Ulvales) and *R. riparium* (Roth) Harvey (order Cladophorales) were grown in sterile PES medium in a climate chamber at 9 °C and 20–25 μmol photons m^{-2} s^{-1} (L58 W/830 Daylight, Osram, München, Germany) and a 16/8 h light/dark regime in aerated 1 L glass beakers. Stock cultures of both isolates were kindly provided by Prof. Wiencke from the Alfred-Wegener Institute in Bremerhaven (Isolate numbers: 1086 for *U. clathrata* and 1118 for *R. riparium*; both from Disco Island, Greenland). Hereafter, these species will be referred to as *Ulva* and *Rhizoclonium*.

2.2. Dose response and recovery experiments in Ulva and Rhizoclonium

Thalli of the two species were simultaneously exposed to artificial UVB radiation produced by fluorescent tubes (TL40/12RS, Philips, Amsterdam, Netherlands) in a growth cabinet (GroBanks, CLF plant climatics, Emersacker, Germany) at 9 °C. In the dose response experiments (experiments D1-4) five different fluence rates ranging from 0 (=control samples) to 10 W m^{-2} UVB_{BE} (weighted after [25]) were applied for 1 h. The UVB fluorescence tubes also emit a constant proportion of visible light which depending on UVB irradiance varied between 2 and 15 μmol photons m^{-2} s^{-1} as measured with a quantum sensor (LI-190, LI-COR, Lincoln, Nebraska, USA). We also weighted the applied UVB irradiances with the weighting function for DNA damage in alfalfa seedlings of Quaite et al. [62] as it was formulated by Musil [52]. This gave virtually the same results and we only show the results from the weighting with the Ghetti spectrum. As the employed lamps emit a spectrum enriched in short wavelength UVB compared to daylight, 1 W m^{-2} weighted UVB from the fluorescence tubes equals 3.15 W m^{-2} unweighted solar UVB. For each species and each UVB irradiance level two open Petri dishes were covered with WG 295 glass filters (Schott, Mainz, Germany) and two Petri dishes with control samples were kept below a UV-blocking Plexiglas filter (GS 321, Röhm, Darmstadt, Germany). The thalli were carefully spread out homogenously in the Petri dishes to minimize self-shading effects. For DNA damage analysis four subsamples taken from each Petri dish were frozen in liquid nitrogen immediately after the exposure and subsequently stored at −85 °C until DNA extraction. Thereafter, the optimal quantum yield of PS II (F_v/F_m) was determined for each treatment using an Imaging PAM chlorophyll fluorometer (Walz GmbH, Effeltrich, Germany). After 20 min predarkening F_0 was assessed with a measuring light between 0.3 and 1.7 μmol photons m^{-2} s^{-1} and F_m with a saturation pulse of >2200 μmol photons m^{-2} s^{-1}.

In a second set of experiments the repair of DNA damage and the recovery of PS II efficiency after 1 h of moderate UVB treatment were investigated under low white light (F15W/35 white, General Electrics, Budapest, Hungary, 10–15 μmol photons m^{-2} s^{-1}) and at 9 °C. UVB exposure was conducted as described above. This time 12 Petri dishes per species were irradiated uniformly and afterwards placed in a second temperature controlled light cabinet for recovery (Type 3000, Rumed, Laatzen, Germany). At different time points two randomly chosen Petri dishes per species were removed from the cabinet and from each dish again four subsamples were frozen in liquid nitrogen. Samples for DNA analysis were taken before the start of the UV exposure (control samples) and at five different time points during recovery up to 48 h after UVB (experiments R1-4/5). For the determination of repair rates of PS II the same set-up as for removal of DNA damages was used but