UVB-induced DNA damage and its repair in marine macroalgae from Kongsfjorden (Svalbard)

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Introduction

Stratospheric ozone depletion has been detected over the Arctic when light returns to this region in early spring (WMO 1998, Dahlback 2002). Although the spatial and temporal scale of this phenomenon is not comparable to the Antarctic ozone hole, prospects of increasing ultraviolet-B radiation (UVBR, 280-315 nm) have raised concern over the potential impact on the productive Arctic marine ecosystem. Macroalgal vegetation that grows close to the water surface can experience high UVBR (Franklin and Forster 1997). UVBR is considered to be harmful because it causes direct damage to organic molecules such as DNA and indirectly increases the production of reactive oxygen species (Setlow et al. 1963, Rijstenbil et al. 2000). Approximately 70-90% of UVB induced DNA damage consists of cyclobutane pyrimidine dimers (Mitchell and Nairn 1989, Dany et al. 2001). These lesions interfere with processes that are crucial for cell function by obstructing DNA expression and replication (Sauerbier and Hercules 1978, Draper and Hays 2000). UV-induced DNA damage has also been linked to reduced growth and survival in marine algae (Poll et al. 2001, Buma et al. 2003). It has been found that ambient UVBR at the water surface can depress the growth of Arctic macroalgae, when compared to PAR exposed plants (Makarov 1999, Aguilera et al. 1999). Although algae have evolved various UV-tolerance mechanisms that allow them to survive harmful UVB effects, variable responses to UVB exposure have been observed among algal species (Karentz et al. 1991a, b, Poll et al. 2001). This variation is presumably caused by differences in the occurrence and effectiveness of their UVB-tolerance mechanisms. Some macroalgae produce polyphenolic compounds and mycosporine like amino acids, which absorb UV radiation and therefore provide protection for sensitive components (Cockell and Knowland 1999). In addition, algae deploy an array of enzymatic repair pathways that remove reactive oxygen species (Aguilera et al. 2002). To remove UVB-induced DNA damage, macroalgae appear to possess the same repair pathways as higher plants. Apart from versatile DNA repair pathways such as nucleotide excision repair, macroalgae probably use light dependent photolyase enzymes that specifically remove UVB-induced DNA damage (Pakker et al. 2000a, b). Although the production of UV-absorbing compounds has been studied in considerable detail, relatively little is known on the active repair of UVB-induced DNA damage in Arctic macroalgae. Low CPD repair activity could make these algae vulnerable to increases in UVBR. In this contribution, we describe experiments that were designed to determine macroalgal sensitivity to UVB-induced DNA damage. To this end, CPD accumulation and repair were tested in field collected Arctic macroalgae, whereas DNA dosimeters were used to assess macroalgal exposure to summertime UVBR.

Method

The experiments were performed in Ny-Ålesund, Svalbard (78° 55.5' N, 11° 56.0' E) in the summer of 2001. Experimental details were published elsewhere (Poll et al. 2002). In short, algae were obtained from Kongsfjorden between 6 and 13 m depth by SCUBA diving and kept overnight in running seawater under low irradiance. Algal fragments of *Laminaria saccharina* (L.) Lamour, *Phycodrys rubens* (L.) Batters, *Palmaria palmata* (L.) Kuntze, *Devaleraea ramentacea* (L.) Guiry, *Coccotylus truncatus* (P.) Wynne and Heine, *Odonthalia dentata* (L.) Lyndberg and *Monostroma arcticum* (Wittrock) were briefly exposed to high artificial UVBR for CPD induction (45 min, 2814 J m^{-2} weighted with Setlow's DNA damage action spectrum). Afterwards, CPD removal was monitored over time in the presence of PAR (14 W m^{-2}) and UVA (9 W m^{-2}). In addition, we tested if CPD accumulation was detectable under natural irradiance conditions. Small fragments of *P. rubens* and *P. palmata* were exposed to unfiltered sunlight just below the water surface for 4 h around noon, whereas PAR exposed fragments served as control. All samples were preserved on silica gel and analyzed for CPDs as described in Poll et al. (2001). Furthermore, DNA dosimeter (Boelen et al. 1999) exposures and spectro-radiometer derived UVB doses were correlated to estimate the maximal CPD concentration that would be induced after 24 h exposure of bare DNA in Svalbard. In addition, DNA dosimeters were repeatedly exposed for two days at several depths in Kongsfjorden to determine attenuation of the wavelengths that cause DNA damage.

Results and discussion

Most algae showed fast repair of accumulated CPDs when challenged for 45 minutes with a UVB dose that would require 8 days of sunshine at highest solar angle in Svalbard (Fig. 1). Around 20 to 75 minutes after CPD accumulation, repair was observed in *P. palmata*, *D. ramentacea*, *L. saccharina* and *P. rubens*, with ~10% of the CPDs remaining after 5 h.

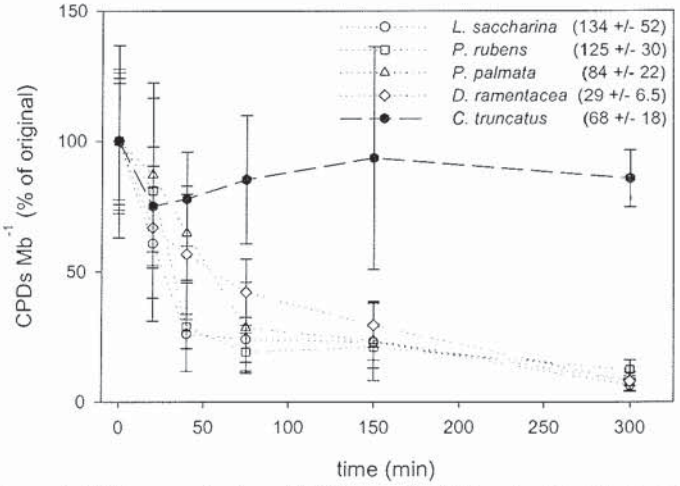

Figure 1. CPD concentrations (CPDs per Mb, 10^6 nucleotides) in *L. saccharina*, *P. rubens*, *P. palmata*, *D. ramentacea* and *C. truncatus* after 45 min of high UVBR, and 20, 40, 75, 150 and 300 min of recovery under PAR and UVAR. CPDs are expressed as a percentage of the initial concentration after 45 min UVBR. Initial CPD concentrations (+/- sd) are indicated in bracelets. Six replicates were analyzed for each species.

Despite the differences in accumulated CPD concentrations after the UVB treatment (ranging from 29 CPDs Mb^{-1} for *D. ramentacea* to 134 CPDs Mb^{-1} in *L. saccharina*, Fig. 1), no significant differences in repair rates could be observed between these species. The differences in CPD accumulation may originate from morphological features and the presence of UV-absorbing compounds, which influence the exposure of the algal DNA. Morphology may be the most important determinant in these experiments because the algae were collected from depths where they contain low concentrations of UV-absorbing compounds (Karsten et al. 1999, Karsten and Wiencke 1999). Experiments with *P. palmata* indicated that repair was light dependent, since no repair was observed in darkness (not shown).

In contrast, no efficient repair was observed after 5 h of recovery in *C. truncatus* (Fig. 1) when challenged by high CPD accumulation, whereas low repair rates were also found in *M. arcticum* and *O. dentata* (not shown). This may point to down regulation of CPD repair under the low (PAR) irradiance present at the collection depth, or to differences in the condition of the tested algae. In terrestrial plants, CPD repair in the form of photolyase activity is regulated by irradiance quality and quantity (Hada et al. 1999, 2001, Ries et al. 2000). Furthermore, photolyase levels differ between tissue types and appear to be adjusted to the concentration of UV-absorbing compounds (Waterworth et al. 2002, Hada et al. 2003). However, it is unknown to what extent Arctic macroalgae regulate CPD repair responses. Apparently, CPD repair was not linked to the distribution pattern of the tested algae as it was observed in deep water and upper subtidal species alike. Probably, the ability to repair UV-induced DNA damage is a feature that is shared by all macroalgae.

Despite the low summertime UVB irradiances, exposure to 4 h of sunlight at noon produced significant CPD accumulation in *P. rubens* (Fig. 2).

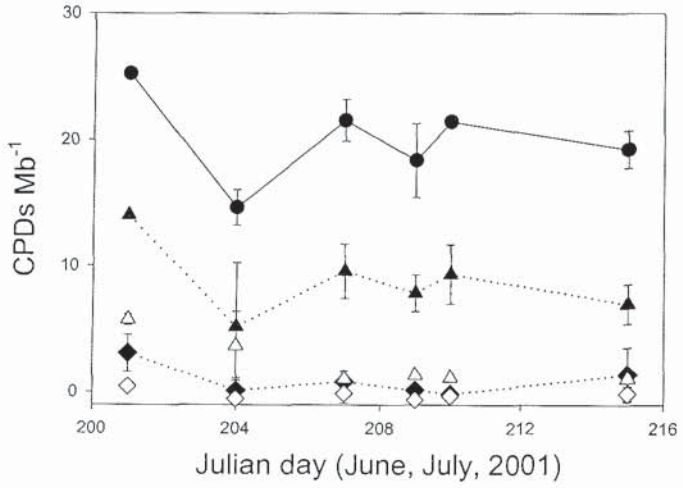

Figure 2. CPDs in DNA dosimeters (circles), *P. rubens* (triangles) and *P. palmata* (diamonds) after exposure to 4 h of sunlight around noon just below the water surface on six days in the summer of 2001. Open symbols show the CPDs in PAR exposed samples. Mean and standard deviation are shown for two dosimeters and six algal fragments.

For *P. rubens*, accumulated CPDs correlated positively with CPDs measured in simultaneously exposed DNA dosimeters (R^2 = 0.86). The rapid bleaching of red pigments under UV exposure and also UV exclusion treatments indicated that the high irradiance levels were extremely stressful for this alga. Consequently, stress caused by high PAR and UVAR may have interfered with the CPD repair response. Less CPD accumulation was observed in *P. palmata*, which can inhabit shallower habitats than *P. rubens*. Although exposure close to the water surface was unnatural for both algae, these experiments show that CPD accumulation is possible in the upper part of the water column in Svalbard.

By correlating dosimeter and spectro-radiometer data it was estimated that maximally ~ 53 CPDs Mb^{-1}can accumulate in bare DNA when exposed for 24 hours just below the water surface in Svalbard (406 J m^{-2}, weighted daily dose (Setlow), Björn and Murphy 1985, data not shown). This value is 20 times lower when compared to those of tropical regions, where up to 1000 CPDs Mb^{-1} can accumulate over the day (Regan et al 1992, Jeffrey et al. 1996, Boelen et al. 1999). The DNA damaging irradiances that reach high latitudinal regions like Svalbard are reduced by reflection and attenuation in the water column before they reach the macroalgal vegetation. Therefore, actual exposure of upper subtidal algae will strongly depend on the attenuation of UVBR in the water column. The DNA dosimeter incubations revealed that the 1% depths for DNA damage ranged between 4 and 8 m for the examined period (Table 1, Fig. 3).

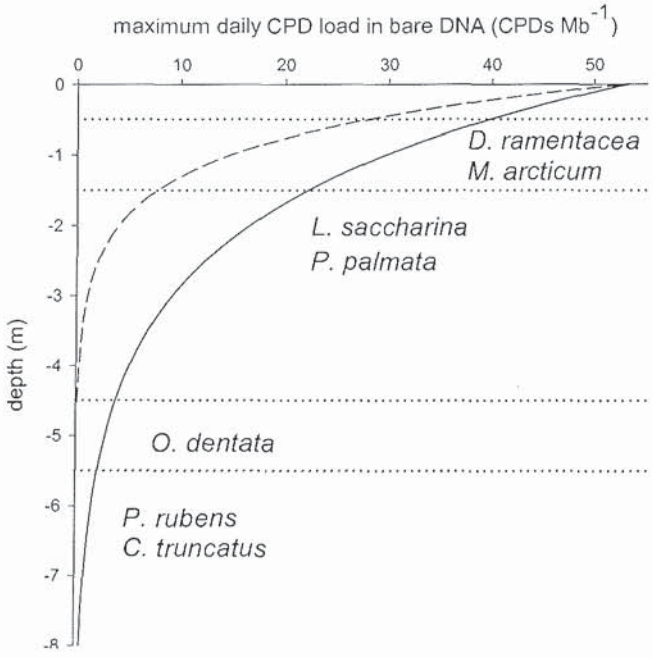

Figure 3. Attenuation of the maximum daily CPD load in DNA dosimeters in Kongsfjorden (Svalbard), calculated for the minimum (line) and maximum (dashed line) attenuation as derived from dosimeter incubations at several depths. The upper vertical distribution boundaries of several species that inhabit Kongsfjorden are indicated with horizontal dotted lines.

Table 1. Diffuse attenuation coefficients (K_d) and 1% depths for DNA damage in Kongsfjorden (Svalbard) calculated from dosimeter data for several time intervals in June and July (2001).

Date (2001)	K_d (m^{-1})	1% depth (m)
13-16 June	0.58	7.94
16-18 June	0.66	6.98
18-20 June	0.74	6.22
20-24 June	0.68	6.77
26-28 June	1.28	3.60
29 June-2 July	1.24	3.71
2-7 July	1.17	3.94

In Kongsfjorden, attenuation is strongly influenced by the influx of sediment rich melt water, as has been described previously (Hanelt et al. 2001). The attenuation of UVBR was much stronger than that found for the open ocean (Boelen et al. 1999). Nevertheless, the dosimeter data confirm that upper subtidal species such as *D. ramentacea* are potentially exposed to DNA damaging wavelengths in the upper part of their vertical distribution range in summer, whereas deep subtidal species like *P. rubens* never receive significant short wavelength radiation (Fig. 3). However, it is uncertain to what extent CPDs are actually formed in the DNA of upper subtidal species like *D. ramentacea*, *P. palmata* and *L. saccharina*, because they produce UV-absorbing compounds in response to the high irradiance present in shallow habitats that may protect their DNA (Karsten et al. 1999, Karsten and Wiencke 1999, Pavia et al. 1997). Consequently, upper subtidal algae have a much higher UV-screening capacity than their low light adapted equivalents that were used in these experiments. Thus, due to the presence of efficient repair and prevention mechanisms in the algae and the relatively low UVB irradiances in the water column, we consider UVB-induced DNA damage to be a minor stress factor for macroalgal vegetation in Kongsfjorden, Svalbard.

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