The Response of Green Alga *Spirogyra* sp. to Different Levels of UV-B Radiation

By

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With 2 Figures

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Summary

GERM M. 2005. The response of green alga *Spirogyra* sp. to different levels of UV-B radiation. - Phyton (Horn, Austria) 45 (2): 173 – 182, 2 figures. - English with German summary.

The response of the green alga *Spirogyra* sp. to different radiation environments was investigated. Samples from high elevation lakes (1669–2150 m a.s.l.) were cultured in the Ljubljana Botanical garden (320 m a.s.l.) and exposed to different levels of UV-B radiation. Biochemical and physiological analyses were made on the lake samples as well as on cultured algae. The level of UV screening compounds decreased after transferring the algae from the lakes to the botanical garden and then increased with enhanced UV-B radiation. Respiratory potential was slightly higher in UV-B treated algae. Enhanced UV-B radiation did not affect the amount of photosynthetic pigments.

Zusammenfassung


Es wurde die Reaktion der Grünalge *Spirogyra* sp. in Umgebungen mit verschiedener Strahlung untersucht. Proben aus Seen von großer Seehöhe (1669–2150 m)

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Introduction

Stratospheric ozone depletion is associated with an increase in biologically harmful solar ultraviolet radiation, which modifies the metabolic activity of terrestrial and aquatic plants (KARSTEN & al. 1998). UV-B radiation (280–320 nm) is reported to increase by 6% to 8% (MADRONICH 1993) up to 20% (BLUMTHALER & al. 1993) per 1,000 m of elevation. In addition, lakes situated above the tree line generally have very high water transparency for UV due to their low concentration of particulate and dissolved matter (SOMMARUGA & PSENNER 1997). Benthic algae play an important role in primary production, being the base of the food web for herbivores and dentritivores, as well as nursery areas for juvenile animals (KARSTEN & al. 1998). The capability for acclimation of photosynthesis to changing radiation environment is an important prerequisite for macroalgae, especially in areas with high natural radiations. SANTAS & al. 1998 concluded that the abundance of certain filamentous algae near the surface may be due partly to their higher UV-B tolerance – a competitive advantage over fast growing species with efficient reproduction and/or chemical defence, but lower UV-B tolerance.

UV radiation causes damage to molecular targets such as DNA, RNA, and proteins (SCHOFIELD & al. 1995, van de POLL & al. 2001). The primary target of UV-B is the D1 protein in the reaction centre of photosystem II (PSII) (HÄDER & al. 2001) and the CO₂ fixing enzyme RubisCO (ALLEN & al. 1997). Various mechanisms for counteracting UV damage have been studied, such as the ability to repair UV-induced damage by excision repair and photoreactivation (EKER & al. 1990) and the accumulation of detoxifying enzymes for eliminating UV-induced oxygen radicals (AGULIERA & al. 2002). An important strategy of sessile autotrophs that inhabit shallow water environments is to reduce UV photodamage by intracellular synthesis and accumulation of UV absorbing compounds (WOOD 1989). Additional supplies of energy, from the respiratory process, are necessary to establish protective mechanisms, such as synthesis of component materials (GULMON & MOONEY 1986). Measurement of the terminal electron transport system (ETS) activity of mitochondria is a good tool for estimating the respiratory potential of organisms (TÖTH & al. 1994).
Very few studies have focused on the comparison of physiological characteristics of algae in their natural environment, in culture and following exposure to enhanced UV-B radiation in outdoor experiments. We tested the tolerance of algae to different radiation environments and their response strategy. The results should be helpful in predicting the consequences for certain macroalgae in shallow, alpine lakes under the changes in UV-B radiation in the near future. The peak in UV-B radiation is supposed to be around year 2000 with a decline toward 2065 (NILSSON 1996, OLSSON 1999).

Abbreviations: AC – absorbing compounds; a.s.l. – above sea level; chl – chlorophyll; DM – dry mass; ETS – electron transport system; INT – iodo-nitro-tetrazolium-chloride; PAR – photosynthetically active radiation; PSII – photosystem II; UV – ultraviolet; UV-A – ultraviolet-A; UV-B – ultraviolet B.

Material and Methods

Plant Material and Growth Conditions

The samples of algae were taken from just below the surface of two transparent lakes, Upper Križ Lake (2150 m a.s.l.) and the Fifth Triglav Lake (1669 m a.s.l.), analysed and then cultured outdoors (Botanical garden, University of Ljubljana: altitude 320 m, 46°35'N, 14°55'E). Effective UV-B \( B_{BE} \) radiation on the Alpine lakes and in Ljubljana was calculated according to the model of BJÖRN & TERAMURA 1993, based on the generalised plant action spectrum of CALDWELL 1971. UV-B \( B_{BE} \) is usually used because different wavelengths do not affect biomolecules and biological processes to the same extent. Thus a number of different UV-B action spectra or weighting functions have been described. Weighting implies multiplying the irradiance by the weighting function, as the estimation of sensitivity for single wavelength band, and then integrating (summing) over all relevant wavelengths (BJÖRN 1999). Algae were transferred from the lakes to the Botanical garden three times in July 2002, and were cultured in plastic containers of 30 cm x 75 cm x 45 cm filled with stabilised tap water. Analyses were made 14 days after culturing the algae. Data from the natural environment (lakes) are indicated by “N” (natural) in graphs and in the Table. An UV-B supplement system was designed as described by BJÖRN & TERAMURA 1993. Three different UV-B radiation treatments were performed: (i) enhanced UV-B radiation simulating 17 % ozone depletion (UV-B(+)), using Q-Panel UV-B 313 (Cleveland, OH, USA) lamps filtered with cellulose diacetate filters, which cut out the UV-C range (wavelengths lower than 280 nm), (ii) reduced level of UV-B radiation (UV-B(−)) using Mylar foil, which cuts out the wavelengths below 320 nm (GEHRKE & al. 1996) and (iii) ambient radiation with Q-Panel UV-B 313 (Cleveland, OH, USA) lamps filtered with Mylar foil, to correct for the effects of the UV-A radiation (control). So the difference between control and UV-B(−) was, that control had he same lamps as UV-B(+), which were wrapped in Mylar foil to filter out UV-B radiation, while UV-B(−) had no lamps.

The systems were timer controlled. The doses simulating 17 % ozone depletion were calculated and adjusted weekly using the programmes of BJÖRN & MURPHY 1985 based on the generalized plant action spectrum (CALDWELL 1971). During the ex-
periment, solar irradiance was measured in three wavelength bands (UV-B, UV-A, and PAR) using ELDONET (Real Time Computer, Möhrendorf, Germany). Doses were calculated on hourly and daily bases for each channel and stored simultaneously on a computer.

Photosynthetic Pigments

Chlorophyll (chl) a and carotenoids were determined following the procedure described by Jeffrey & Humphrey 1975. Weighed thalli of algae were homogenized in 8 cm$^3$ of 90 % (v/v) acetone and centrifuged (19 000 x g, 3 min, 4 °C) in a top refrigerated centrifuge (2K15, Sigma, Osterode, Germany). The chl a and carotenoid contents were calculated per sample dry mass (DM) from absorbances at 647, 664, 480 and 750 nm measured with a UV/VIS Spectrometer System Lambda 12 (Perkin-Elmer, Norwalk, CT, USA).

UV Absorbing Compounds

Methanol soluble UV-B and UV-A absorbing compounds (AC) were extracted from fresh homogenized material with methanol : distilled water : HCl (37% v/v) = 79 : 20 : 1 (v/v/v) according to Mirecki & Teramura 1984. After 20 min incubation the samples were centrifuged (1 600 x g, 10 min, 10 °C). The supernatants were scanned in the range from 280 to 320 nm for UV-B and from 320 to 400 nm for UV-A absorbing compounds at intervals of 1 nm. The absorbance values per sample dry mass (DM) were integrated to estimate the content of substances absorbing in the UV-B and UV-A ranges. The mean DM were 0.0052 g, 0.0048 g, 0.0043 g and 0.0044 g for the lake samples, UV-B(-), control and UV-B(+), respectively.

Terminal Electron Transport System Activity

Terminal electron transport system (ETS) activity of mitochondria was assayed as described by Packard 1971. Determination of ETS activity is based on reduction of the artificial electron acceptor iodo-nitro-tetrazolium-chloride (INT) and on the spectrophotometric measurement of formazan production rate, which is directly related to oxygen consumption of the investigated tissue. Samples were homogenized in ice cold 0.1 M sodium phosphate buffer (pH = 8.4) containing 0.15% (w/v) polyvinyl pyrrolidone, 75 μM MgSO$_4$, and 0.2% (v/v) Triton-X-100, sonicated (40 W, model 4710, Cole-Parmer, Vernon Hills, IL, USA) and centrifuged (8 500 x g, 4 min, 0 °C). Substrate solution (0.1 M sodium phosphate buffer (pH = 8.4), 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100), and 20 mg 2-p-iodo-phenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride (INT) in 10 ml of bidistilled water, were added to triplicates of the supernatant and incubated at 20 °C for 40 min. The reaction was stopped with 0.5 ml solution of formaldehyde and phosphoric acid 1 : 1. The absorbance of formazan was measured at 490 nm. ETS activity was measured as the rate of INT reduction, which was converted to the amount of oxygen (Kenner & Ahmed 1975) utilized per DM unit of thalli per hour.

Statistical Analyses

Each measurement was repeated five times; mean values and standard deviations were calculated. Results are presented as the mean values of all experiments.
The significance of differences between different levels of UV-B radiation was tested by Student t-test where appropriate. Differences at the 5% level probability were accepted as significant.

Results and Discussion

The higher levels of UV-B and UV-A AC were observed in *Spirogyra* grown in the natural environment. When transferred to research plots, the levels decreased; but the amount of UV-B AC increased again with increasing UV-B dose, although did not reach those in the natural environment (Fig. 1). These results are in good relation to UV-B radiation in the Alps, which is higher than that under enhanced UV-B radiation in the Botanical garden in Ljubljana. The ability of *Spirogyra* to synthesize protective UV AC in response to different levels of UV-B is of great ecological importance, given the variable light environment in Alpine aquatic ecosystems. Thus, the algae at higher altitudes are protected from strong UV-B radiation by high levels of screening substances. Beside UV, higher intensities of PAR also supported the synthesis of UV AC in this alga. Our results are in line with those of Post & Larkum 1993 who observed high levels of UV absorbing compounds in *Prasiola*, especially in the top layers.
of mats in summer. On the basis of transplantation experiments and analysis of samples from different depths of water, Karsten & al. 1998 concluded that UV-B has a strong effect on the synthesis of UV sunscreens in macroalgae. Larkum & Wood 1993 observed that deep water benthic algae were more sensitive than intertidal benthic algae, due to the adaptive capability of the latter to accumulate UV sunscreens. The levels of UV AC from Spirogyra are comparable to those in vascular aquatic plants (Germ & al. 2002b). However, evidence has been reported that extract from green algae do not have strong absorption in the UV wavelengths (Karentz & al. 1991).

The increased amount of UV absorbing substances in Spirogyra exposed to enhanced UV-B radiation provides further evidence for this being a photoprotective mechanism. The amount of UV AC was higher than that reported for other green algae, as well as in Dasycladus vermicularis (Pérez-Rodrigues & al. 1998).

The pattern of the increasing and decreasing levels of UV-B AC and UV-A AC and ETS activity is similar (Figs. 1,2). The synthesis of UV screening substances demands an additional supply of energy, which was provided by higher respiratory potential. The synthesis of these substances is energetically costly, which is justified if the damage due to UV-B is bigger than metabolic cost of production (Gaberščik & al. 2002). The in-
increased ETS activity of algae also reflects the energetic cost of repair mechanisms that counteract the damage at the molecular level. Ziska & al. 1992, who studied the physiological sensitivity of plants as a function of elevation, found that respiration increased with increasing UV-B radiation in the high elevation population, which indicates that additional energy is needed for repair. The effect of UV-B on the respiration of aquatic primary producers has been studied (Larkum & Wood 1993, Post & Larkum 1993, Hader & al. 1996, Kinzie & al. 1998, Heraud & Beardall 2002), but little is known about its influence on the ETS activity of algae (Ferreira & al. 1997, Gabersck & al. 2002, Germ & al. 2002a, 2004). The ETS activity of phytoplankton has been shown to increase under enhanced UV-B radiation, in accordance with the present findings.

The amount of chl a and carotenoids were higher in the samples from the natural environment. No significant changes were observed in the amounts of chl a and carotenoids, as the result of artificially enhanced UV-B radiation on Spirogyra (Table 1). UV-B has been reported to induce decrease of photosynthetic pigments (Bischof & al. 1998), while no effect of UV-B on chl a was observed by Sundbäck & al. 1997 and Jahnke 1999. De Lange & al. 2000 reported a negligible effect on chl a levels in UV-B treated cells of Selenastrum, although it was found to increase in UV-B treated Scenedesmus quadricauda and Selenastrum capricornutum (Germ & al. 2002a, 2004).

Table 1. Mean values and standard deviations of chlorophyll a and carotenoids in Spirogyra grown under different levels of UV-B radiation.

<table>
<thead>
<tr>
<th>Environment/ treatment</th>
<th>Chlorophyll a (mg/gDM)</th>
<th>Carotenoids (mg/gDM)</th>
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<tbody>
<tr>
<td>Natural</td>
<td>5.49 (2.31)*</td>
<td>2.54 (0.99)*</td>
</tr>
<tr>
<td>UV-B(-)</td>
<td>2.30 (0.89)</td>
<td>1.27 (0.49)</td>
</tr>
<tr>
<td>Control</td>
<td>2.00 (0.45)</td>
<td>1.05 (0.21)</td>
</tr>
<tr>
<td>UV-B(+)</td>
<td>1.91 (0.73)</td>
<td>0.93 (0.28)</td>
</tr>
</tbody>
</table>

Legend: natural – samples from the lakes, UV-B(-) reduced, and UV-B(+) enhanced UV-B radiation in outdoor experiments. * indicates significantly different values (N = 15, p < 0.05) between samples from the lakes, and samples from the control, reduced and enhanced level of UV-B radiation.

The ability to respond to UV-B radiation with increasing synthesis of screening compounds indicates the physiological plasticity and resistance of Spirogyra. The findings from the present experiments provide useful information since results from outdoor (semi-natural) experiments were combined with data obtained in the lake (natural) conditions.
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