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Original Paper

Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae

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Abstract. The activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX) and catalase (CAT), as well as the content of the antioxidant compound ascorbic acid, were determined in five green, seven red and ten brown macroalgal species from the Kongsfjord (Spitsbergen, Svalbard, Norway). In general, higher antioxidant enzyme activities and a higher content of ascorbic acid were measured in green algae in comparison to red and brown algae. Species from the eulittoral and upper sublittoral (*Acrosiphonia penicilliformis*, *Monostroma arcticum*, *Chaetomorpha linum*, *C. melagonium*, *Devaleraea ramentacea*, *Palmaria palmata*) showed higher antioxidant enzyme activities compared to species from the lower sublittoral, indicating a more efficient biochemical protection in algae exposed to higher stress conditions in the field. The activity of GR was stimulated by artificial ultraviolet radiation in the green alga *M. arcticum*, and in the red algae *Coccotylus truncatus* and *Phycodrys rubens* after 84 h under continuous exposure. GR activity was even higher when the UV exposure was followed by incubation in darkness for 24 h, indicating a higher elimination rate of toxic oxygen radicals under these conditions. *D. ramentacea*, *Palmaria palmata* and *A. penicilliformis* did not show any significant effect of UV radiation on CAT, APX and SOD activities after 8 days of culture under laboratory conditions. However, a significant reduction in activities of GR and SOD was observed in *A. penicilliformis* when solar UV radiation was cut off by selective filter foils in the field, indicating a

lower oxidative stress in the absence of UV radiation. Overall, the ecological success of macroalgae in the eulittoral and upper sublittoral is supported by an enhanced oxygen-reactive scavenging system, allowing fast acclimation to the changes in environmental radiation conditions.

Introduction

Studies of the ability of living organisms to cope with enhanced levels of ultraviolet radiation become more and more important due to the increasing depletion of stratospheric ozone. Recent data show a dramatic trend of ozone depletion over the Antarctic regions, with a temporal decrease in springtime below 25-30% of the undisturbed conditions (NASA http://toms.gsfc.nasa.gov/ozone/ozone.html). Strong reduction of the stratospheric ozone is now also evident in the northern hemisphere (Ott and Amanatides *1994*; Schulz et al. *2001*), and predictions indicate a gradual increment of ultraviolet (UV) radiation in the northern polar regions similar to the southern hemisphere (Stolarski et al. *1992*).

>Accurate information is needed to assess the potential effects in organisms caused by UV radiation reaching the earth's surface. The effects of UV radiation on growth and other physiological features, such as damage in DNA, RNA, proteins and photosynthesis, in a range of higher and lower plants including phytoplankton are relatively well documented (Aguilera et al. *1999a*, *b*; Bischof et al. *2000*; Buma et al. *1995*; Clendennen et al. *1996*; Figueroa et al. *1997*; Häder and Figueroa *1997*; Karentz et al. *1991*; Smith et al. *1992*; Strid et al. *1990*; Tevini and Teramura *1989*) while investigations on the effects of UV radiation on benthic marine macroalgae are scarce, although this group plays an important ecological role in the marine environment.

Photosynthesis can be damaged due to high photosynthetically active radiation (PAR) or UV radiation, as a result of an overreduction of the photosynthetic electron transport when not enough electrons are drained off by $NADP^+$ to NADPH from reduced ferredoxin of PSI. UV radiation can affect the draining-off system by damaging proteins of the Calvin cycle like Rubisco (Allen *1977*; Bischof et al. *2000*). Thus, in the absence of $NADP^+$, the reduced ferredoxin can also reduce oxygen, leading to superoxide radicals (O_2). Consequently, photosystem II is inactivated by UV and finally damaged due to degradation of the reaction centre proteins, mainly the D1 protein (Aro et al. *1993*; Ohad et al. *1984*). Under such conditions, singlet oxygen ($^1O_2$) can be formed from triplets of chlorophyll of the antenna. Reactive oxygen species can produce lipid peroxidation, damage proteins and have many other harmful effects (Asada and Takahashi *1987*; Fridovich *1986*).

Cellular mechanisms of protection against such toxic oxygen species are essential for the maintenance of photosynthetic activity and other metabolic functions (Allen *1977*; Asada and Takahashi *1987*; Eltsner *1982*; Halliwell *1982*). Plants and algae are equipped with an array of defence mechanisms that eliminate toxic oxygen radicals produced as by-products of photosynthesis and photooxidative events. Superoxide radicals are eliminated by the enzyme superoxide dismutase (SOD), yielding H_2O_2 and oxygen. Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is probably an intracellular precursor for more reactive oxidants, such as hydroxyl radicals. H_2O_2 is deprotonated by the enzyme catalase and by specific scavengers such as ascorbate and glutathione, mediated by ascorbate peroxidase and glutathione peroxidase, respectively. The resulting oxidized reactants, namely monodehydroascorbate and oxidized glutathione, are regenerated via enzymatic reductions by monodehydroascorbate reductase and glutathione reductase, respectively, thereby closing the antioxidant scavening cycle (Polle *1996*).

Studies related to UV-induced photooxidation to the scavenging mechanisms for protection against oxidative damage are rare for macroalgae. Thus, the ability to resist high radiation stress may be one of the major factors controlling vertical macroalgal zonation patterns in communities (Bischof et al.

1998a; Hanelt *1998*), and may be mediated by a higher biochemical potential against oxidative stress. Long-term exposure under UV radiation has been demonstrated to induce the activity of superoxide dismutase and ascorbate peroxidase in microalgae (Lesser *1996a*, *b*; Malanga and Puntarulo *1997*). Activities of antioxidant enzymes were higher in shallow-water coral zooxanthellae than in specimens collected from deeper waters (Shick et al. *1995*). In addition, analysis of the antioxidant spectrum in selected alpine plant species collected at different altitudes proved that the total amount of antioxidants is positively correlated with altitude (Wildi and Lütz *1996*). In one of the few publications on macroalgae, it was postulated that the differential stress tolerance associated with the vertical zonation of different *Fucus* species is strictly related to the antioxidant status of the plant, based mainly on species-specific differences of antioxidant enzyme activities (Collen and Davison *1999a*, *b*).

Despite the potential importance in providing an alternative sink for excessively absorbed radiation energy and their role in scavenging, little is known about the capacity or inducibility of macroalgal antioxidant enzyme systems. The present study was designed to characterize the oxidative stress tolerance in field material of different green, red and brown macroalgae from the Arctic by the analysis of a set of antioxidant enzyme activities and the ascorbic acid content, as well as by the response to UV radiation.

Materials and methods

Algal material and study site

The macroalgal species studied and their depth distribution are listed in Table 1. Plants were collected by scuba divers in summer 1998 at the study site in the Kongsfjord (Ny-Ålesund, Spitsbergen, Norway 78°55.5′ N; 11°56.0′ E) from depths between 0 and 20 m. Algal samples were collected in black bags to avoid exposure to high irradiance during transport. Material for enzymatic activities and for ascorbic acid determination was immediately frozen in liquid nitrogen and kept at -30°C prior to analysis. Samples were kept for at least 48 h under white fluorescent lamps (35 µmol m^{-2} s^{-1}) in running seawater pumped directly from the fjord before laboratory experiments started.

Table 1. Investigated macroalgal species from the Kongsfjord on the Arctic island of Spitsbergen (Svalbard, Norway) and their occurrence in the eulittoral, upper sublittoral (0-3 m) and lower sublittoral (3-20 m) zone according to Svendsen (*1959*) and Klekowski and Weslawski (*1990*)

Species	Habitat
Chlorophyta	
Acrosiphonia penicilliformis (Foslie) Kjellman	Eulittoral-upper sublittoral
Monostroma arcticum Wittrock	Upper-lower sublittoral
Chaetomorpha linum (Müller) Kützing	Upper-lower sublittoral
Chaetomorpha melagonium (F. Weber et Mohr) Kützing	Upper-lower sublittoral
Prasiola crispa (Lightfoot) Meneghini	Eulittoral
Rhodophyta	
Coccotylus truncatus (Pallas) M.J.Wynne & J.N.Heine	Lower sublittoral
Devaleraea ramentacea (L.) Guiry	Eulittoral-upper sublittoral
Palmaria palmata(L.) Grev.	Upper-lower sublittoral
Phycodrys rubens(L.) Batters	Lower sublittoral
Odonthalia dentata(L.) Lyngb	Lower sublittoral
Polysiphonia arctica J. Agardh	Lower sublittoral
Ptilota gunneri P.C.Silva, Maggs & L.M.Irvine	Lower sublittoral
Phaeophyta	
Alaria esculenta (L.) Greville	Lower sublittoral
Chorda tomentosa Lyngbye	Upper-lower sublittoral
Chordaria flagelliformis (O. F. Müller) C. Agardh	Upper sublittoral
Desmarestia aculeata (L.) Lamouroux	Lower sublittoral
Elachista fucicola (Velley) Areschoug	Eulittoral-upper sublittoral
Fucus distichus L.	Eulittoral-upper sublittoral
Laminaria saccharina(L.) Lamouroux	Upper-lower sublittoral
Laminaria solidungula J. Agardh	Lower sublittoral
Laminaria digitata(Huds.) Lamouroux	Upper-lower sublittoral
Saccorhiza dermatodea(de la Pylaie). J. Agardh	Upper-lower sublittoral

For laboratory experiments, approximately 10 g fresh weight (FW) of algae was incubated in 5-l plastic tanks in running seawater at a temperature of 2°C and exposed to 38 µmol m^{-2} s^{-1} PAR provided by one Osram daylight fluorescence tube, 8 W m^{-2} UVA (320-400 nm) and 0.36 W m^{-2} UVB (280-320 nm) provided by two Q-Panel UVA-340 fluorescence tubes (Q-Panel, Cleveland, Ohio). Total PAR+UVA+UVB (295-700 nm) radiation treatment was obtained by covering the tanks with Ultraphan cut-off filter foil (cut-off wavelength <295 nm; Ultraphan, Digefra, Munich, Germany). For the PAR treatment, the tanks were covered with polyester cut-off filter foil (cut-off wavelength <395 nm; Folex, Dreieich, Germany). Radiation measurements were carried out with a Li-Cor LI-190-SB cosine corrected sensor connected to a Li-Cor LI-1000 datalogger (Lambda Instruments, Lincoln, Neb.) for PAR (400-700 nm), and with an RM-21 broad-band UV radiometer (Dr. Gröbel, Ettlingen, Germany).

In a first set of experiments, the red algae, *Coccotylus truncatus*, *Phycodrys rubens* and the green alga, *Monostroma arcticum*, were exposed for 3 days under continuous PAR and PAR+UVA+UVB radiation. Samples were taken at the start of the experiment and after 24 h and 84 h of exposure. In parallel, at the same time, subsamples were taken and maintained in darkness for 24 h under otherwise identical culture conditions, in order to characterize the possible recovery processes in darkness after exposure to UV radiation.

In a second set of experiments, thalli of the red algae, *Palmaria palmata*, *Devaleraea ramentacea* and the green alga, *Acrosiphonia penicilliformis*, were exposed for 8 days under continuous PAR and PAR+UVA+UVB. Parallel to the laboratory experiments, thalli of *A. penicilliformis* were covered in situ by means of an 80 × 80 cm^2 UV transparent Plexiglas plate, wrapped with the 395 nm cut-off filter foil to avoid UVA plus UVB. These samples were compared with subsamples exposed to full solar radiation at the same part of the rocky shore.

Enzyme activities

Samples (0.2-0.3 g FW) of the studied species were ground in liquid nitrogen and extracted with 1-1.5 ml 50 mM potassium phosphate buffer (pH 7.0) containing Complete protease inhibitor cocktail (Boehringer, Mannheim, 2 tablets in 100 ml buffer). Extracts were centrifuged for 15 min at 15,000 rpm at 4°C. Catalase was analysed according to Aebi (*1984*); 10-40 µl extract was added to 810-840 µl potassium phosphate buffer (50 mM, pH 7). The reaction was started by the addition of 150 µl of H_2O_2 solution in phosphate buffer (15 mM final concentration in the cuvette) and followed by monitoring the decrease in absorbance at 240 nm at 20°C for 1-2 min. Catalase activity was calculated by subtracting the non-enzymatic reaction and using an extinction coefficient of 0.0398 mM^{-1} cm^{-1}. Glutathione reductase (GR) was assayed according to Goldberg and Spooner (*1983*); 10-40 µl extract was added to 960-990 µl of a buffer containing 80 mM Tris buffer (pH 8), 1 mM EDTA, 0.1 mM NADPH, and 0.5 mM GSSG, and oxidation of NADPH was followed at 340 nm at 20°C. GR activity was calculated by subtracting the non-enzymatic reaction and using an extinction coefficient for NADPH of 6.22 $mM^{-1}cm^{-1}$. Samples for ascorbate peroxidase (APX) activities were extracted with the same protocol as for the other enzymes with the modification that 0.5 mM of ascorbate was added to the extraction buffer for the stability of the APX (Chen and Asada *1989*). Enzyme activities were assayed according to the same authors and the decrease of absorbance at 290 nm was followed for 1 min after adding 10-40 µl extract to 960-990 µl 50 mM phosphate buffer (pH 7) containing 0.1 mM of H_2O_2, and 0.5 mM ascorbate. All assays were performed at 20°C. APX activity was calculated by subtracting the non-enzymatic reaction and using an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Results for catalase, GR and APX are expressed as units (*U*) of enzyme activity per milligram of total soluble protein [1 *U*=1 µmol substratum (H_2O_2, NADPH and ascorbate, respectively) converted min^{-1}]. SOD was measured using the xanthine oxidase-cytochrome c reduction method (McCord and Fridovich *1969*). In this coupled reaction, SOD inhibits the reduction of cytochrome c by superoxide anions generated from xanthine. The assay mixture (860-1,000 µl) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 10 µM cytochrome c and 50 µM xanthine. Xanthine oxidase (Merck) was added to give an increase of absorbance at 550 nm of 0.025±0.003 min^{-1} at 20°C. Samples (10-50 µl) were added to the reaction mixture and the rate of reduction of cytochrome c was followed spectrophotometrically at 550 nm, and 1 unit of SOD was defined as the amount of enzyme required to inhibit the rate of cytochrome c reduction by 50%.

Ascorbic acid was measured according to Foyer et al. (*1983*). Thalli of 0.2-0.4 g fresh weight were ground in liquid nitrogen and extracted with 1-1.5 ml 100 mM potassium phosphate buffer (pH 5.6) containing 5 mM dithioerythritol (DTE). Extracts were centrifuged for 15 min at 15,000 rpm at 4°C.

The ascorbate content was determined by the disappearance of absorbance at 265 nm after addition of 10 *U* ml^{-1} ascorbate oxidase and 50 µl sample to 925 µl sodium phosphate buffer (100 mM, pH 5.6). Amounts were quantified using a standard curve with 1.25-12.5 µM of pure ascorbate in the reaction mixture.

Protein assay

Total soluble proteins of the crude extract for antioxidant enzyme activities were determined using a commercial Protein Assay (BioRad), based on the Bradford method (Bradford *1976*). Protein content was determined spectrophotometrically at 595 nm and concentrations were calculated compared with a standard of bovine serum albumin (SIGMA).

Statistics

Mean values and their standard deviations were calculated from the different replicates per treatment. Statistical significances of means were tested with a model 1 one-way ANOVA, followed by a multi-range test by Fisher's protected least significance difference (LSD) (Sokal and Rohlf *1995*), and a $P<0.05$ was considered to be statistically significant.

Results

Antioxidant enzyme distribution in Arctic macroalgae

To investigate the protection mechanisms against oxidative stress, a total of 22 species of green, red and brown Arctic macroalgae were analysed for the presence of the activity of superoxide dismutase, glutathione reductase, ascorbate peroxidase and catalase (CAT) (Table 2). Clear differences were found between the three macroalgal groups, with green algae showing in general higher antioxidant enzyme activities than red and brown algae. Independent of the reference parameters tested to express enzyme activity (fresh weight or protein content), these taxon-specific differences remained similar. Maximum SOD activities were found in *M. arcticum* and *A. penicilliformis*, exhibiting values of 1,004 and 674 U mg TSP^{-1}, respectively.

Table 2. Enzymatic activities of superoxide dismutase (*SOD*), glutathione reductase (*GR*), catalase (*CAT*), ascorbate peroxidase (*APX*) and the content of ascorbic acid in different green, red and brown algae from the Kongsfjord (Spitsbergen). Results are expressed as units (*U*) of enzyme activity per milligram of total soluble proteins (*TSP*) where 1 *U*=1 µmol substrate converted min^{-1}. Standard deviations were less than 20% (- not measured)

Species	SOD	GR	APX	CAT	Ascorbate
	(*U*mg TSP^{-1})	(*U*mg TSP^{-1})	(*U*mg TSP^{-1})	(*U*mg TSP^{-1})	(mg gFW^{-1})
Chlorophyta					
Acrosiphonia penicilliformis	674	2.30	0.2	1.0	0.19
Monostroma arcticum	1004	1.58	0.97	27.11	1.63
Chaetomorpha linum	395	0.10	0.778	0.77	0.65
Chaetomorpha melagonium	200	1.54	0.5	30.00	1.57
Prasiola crispa	153	0.10	0.12	3.86	-
Rhodophyta					
Coccotylus truncatus	165	0.08	0.05	9.91	0.27
Devaleraea ramentacea	245	0.32	0.60	15.5	0.2
Palmaria palmata	185	0.22	0.45	4.5	0.43
Phycodrys rubens	94	0.08	0.04	-	-
Odonthalia dentata	109	0.09	0.10	6.24	0.43
Polysiphonia arctica	87	0.06	0.02	7.58	0.18
Ptilota gunneri	122	0.11	0.01	-	0.2
Phaeophyta					
Alaria esculenta	102	0.07	0.05		0.06
Chorda tomentosa	128	0.11	0.07	-	0.75
Chordaria flagelliformis	112	0.10	0.03	-	-
Desmarestia viridis	143	0.10	0.08	-	0.31
Elachista fuciola	191	0.10	0.05	-	-
Fucus distichus	151	0.09	0.07	3.60	0.29
Laminaria saccharina	181	0.18	0.04	7.97	0.17
Laminaria solidungula	142	0.08	0.01	-	traces
Laminaria digitata	68	0.10	0.05	-	traces
Saccorhiza dermatodea	88	0.09	0.06	-	0.58

SOD activities in all species tested were significantly higher ($P<0.05$) compared to GR, APX und CAT. Maximum GR activities were measured again in the green algal group, with the highest value of 2.3 U mg TSP^{-1} found in *A. penicilliformis*. Red and brown algae showed species-specific GR activities ranging from 0.07 to 0.32 U mg TSP^{-1}. Similar results were obtained in APX and CAT activities, and in both cases *M. arcticum* and *Chaetomorpha* species exhibited highest values. *D. ramentacea* showed exceptionally high values of APX and CAT activities, being 6 times higher in APX and almost 2 times higher in CAT compared to the other investigated red algae (Table 2). The internal concentrations of ascorbate in the different species vary from traces in brown algae up to values of 1.63 mg ascorbate gFW^{-1} in *Chaetomorpha melagonium.*

Ultraviolet radiation effects on antioxidant enzymes

Ultraviolet radiation leads to a significant enhancement ($P<0.05$) of the enzymatic activities of GR in laboratory experiments (Fig. 1a, b, c: note the different species-specific scales). Exposure to 8 W m^{-2} UVA and 0.36 W m^{-2} UVB promoted an increase of 22% in GR activity in the green alga *M. arcticum* after 24 h of continuous irradiation compared to the control under PAR radiation (Fig. 1a). GR activity in subsamples kept for 24 h in darkness increased further. After 84 h exposure, GR activity rose under both radiation conditions, especially after exposure to PAR and UV radiation. After 84 h exposure, followed by 24 h of darkness, GR activity was slightly higher ($P<0.05$) in specimens previously exposed to UV radiation.

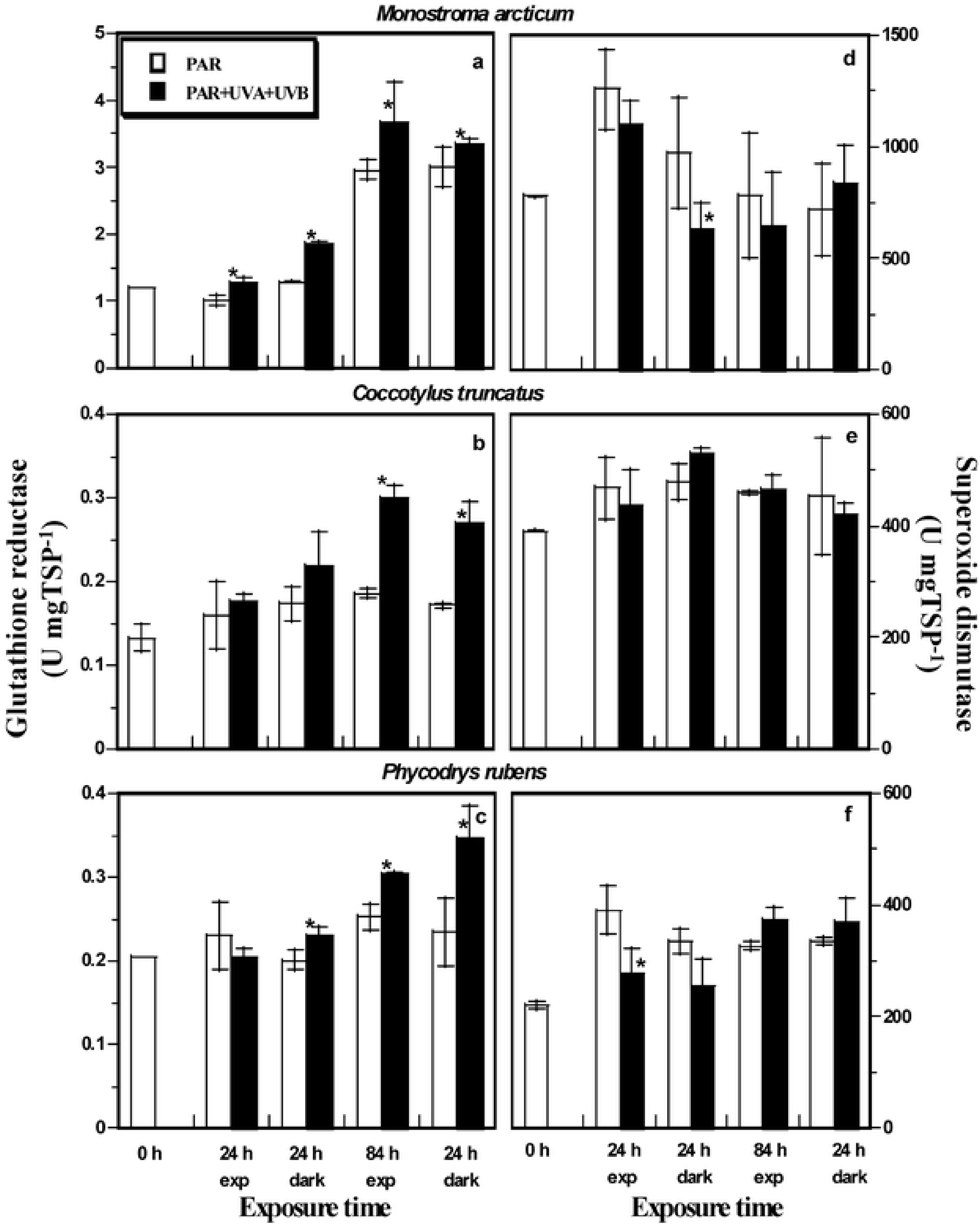

Fig. 1a-f. Influence of exposure under artificial PAR (*grey bars*) and PAR+UVA+UVB (*black bars*) radiation on enzymic activities of glutathione reductase (*GR*) and superoxide dismutase (*SOD*) in the red algae *Coccotylus truncatus* and *Phycodrys rubens* and the green alga *Monostroma arcticum* from Spitsbergen. Plants were exposed for 84 h under continuous irradiation. Subsamples were cultured for 24 h in darkness after 24 and 84 h exposure. Data are given as mean values±SD (*n*=3) and expressed as Units mg^{-1} total soluble proteins (*TSP*). Mean values with different *asterisks* are significantly different (at $P=0.05$) to the control

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In the red algae *Coccotylus truncatus* and *Phycodrys rubens*, 24 h of continuous UV radiation did not result in significant differences of GR activity with respect to the control (Fig. 1b, c). However, after 84 h of continuous UV radiation, a significant increment ($P<0.05$) in GR activity from 0.18 to 0.31 U mgTSP^{-1} for *Coccotylus truncatus* and from 0.25 to 0.32 U mgTSP^{-1} for *Phycodrys rubens* was measured. Moreover, the GR activity increased in subsamples of both species cultured in darkness following the UV exposure.

In contrast to GR activity, SOD in the three algal species studied seemed not to be positively affected ($P>0.05$) at the end of the exposure to the radiation treatments (Fig. 1d, e, f). In *M. arcticum* and *Phycodrys rubens*, UV promoted a significant decrease of SOD activity after 24 h of exposure and after 24 h in darkness ($P<0.05$), but after 84 h exposure and the following 24 h of darkness, no significant difference ($P>0.05$) between the two radiation conditions could be detected. The red alga *Coccotylus truncatus* showed an unchanged SOD under all treatment conditions.

The red algae, *Palmaria palmata* and *D. ramentacea*, were kept for 8 days under continuous UV radiation under the same conditions as described above. For both species, no significant UV effect was observed ($P>0.05$) in CAT, APX and SOD activities (Fig. 2a-f). Although CAT actvities in both plants increased within 8 days, significant differences between the radiation treatments could not be detected (Fig. 2a, d). For APX, an inconsistent activity pattern was observed. After UV exposure, variation was high in both species, with no specific pattern or effect (Fig. 2b, e). In *Palmaria palmata*, SOD activities markedly increased under PAR within the 8 days of exposure, whereas activities under UV radiation were lower (Fig. 2c). In *D. ramentacea*, no significant changes in SOD activities could be recorded (Fig. 2d).

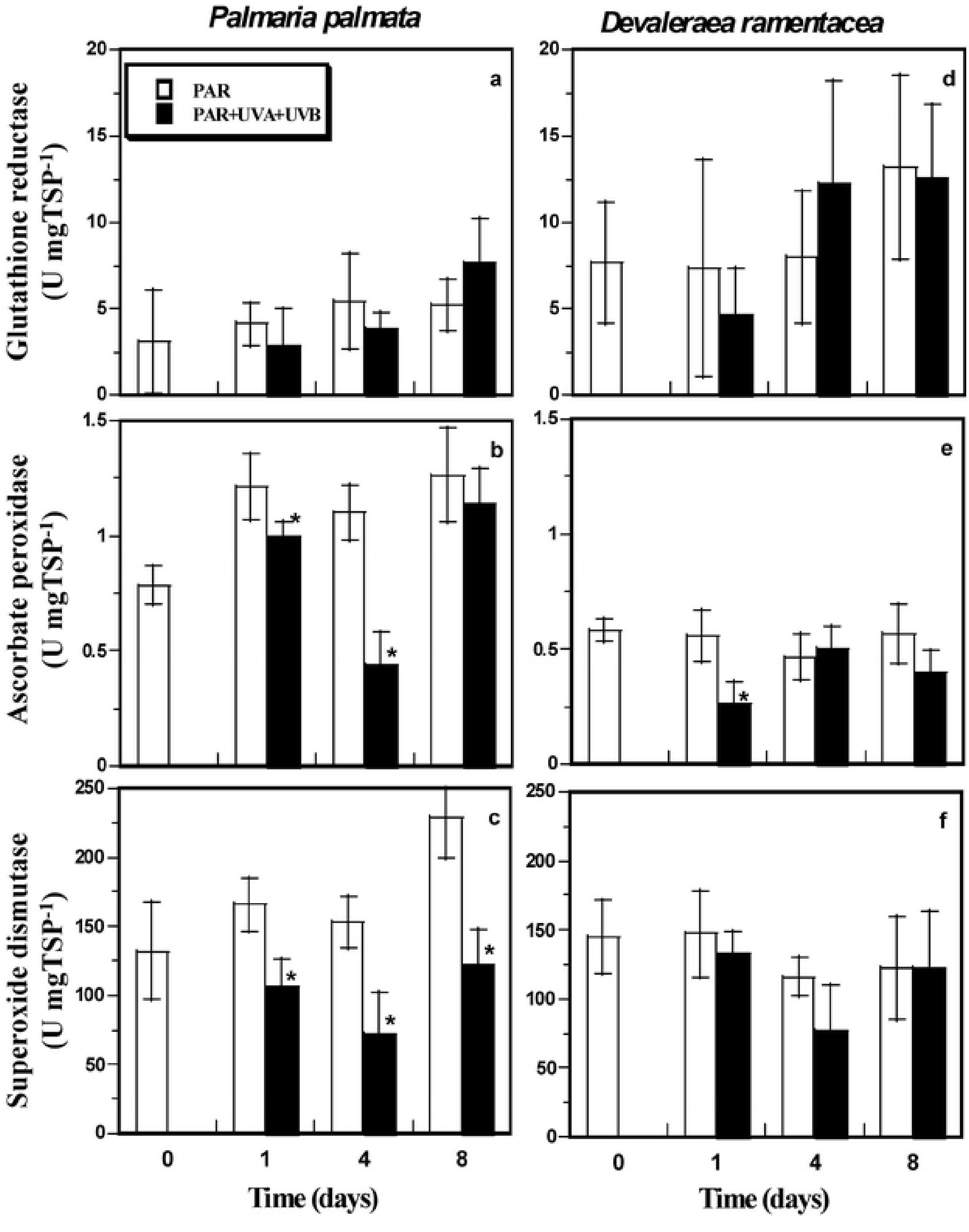

Fig. 2a-f. Influence of continuous exposure under artificial PAR (*grey bars*) and PAR+UVA+UVB (*black bars*) radiation on enzymatic activities of glutathione reductase (GR), ascorbate peroxidase (*APX*) and superoxide dismutase (*SOD*) in the Arctic red alga *Palmaria palmata* and *Devaleraea ramentacea*. Data are given as mean values±SD (*n*=3) and expressed as Units mg^{-1} total soluble proteins (*TSP*). Mean values with different *asterisks* are significantly different (at *P*=0.05) to the control

In a third set of radiation experiments, the eulittoral green alga *A. penicilliformis* was kept for 8 days under continuous UV irradiation under the same laboratory conditions as before (Fig. 3a, b). In this case, no significant differences were observed in GR and SOD activities when the algae were maintained under PAR and PAR+UVA+UVB radiation ($P > 0.05$). In contrast, in the field experiment, algal thalli were exposed in situ to the full solar spectrum and compared with thalli where UVA+UVB was filtered out of the natural solar radiation spectrum (Fig. 3c, d). Samples exposed for 8 days to UV filtered radiation showed a significant decrease ($P < 0.05$) in GR and SOD activity with respect to the fully exposed subsamples.

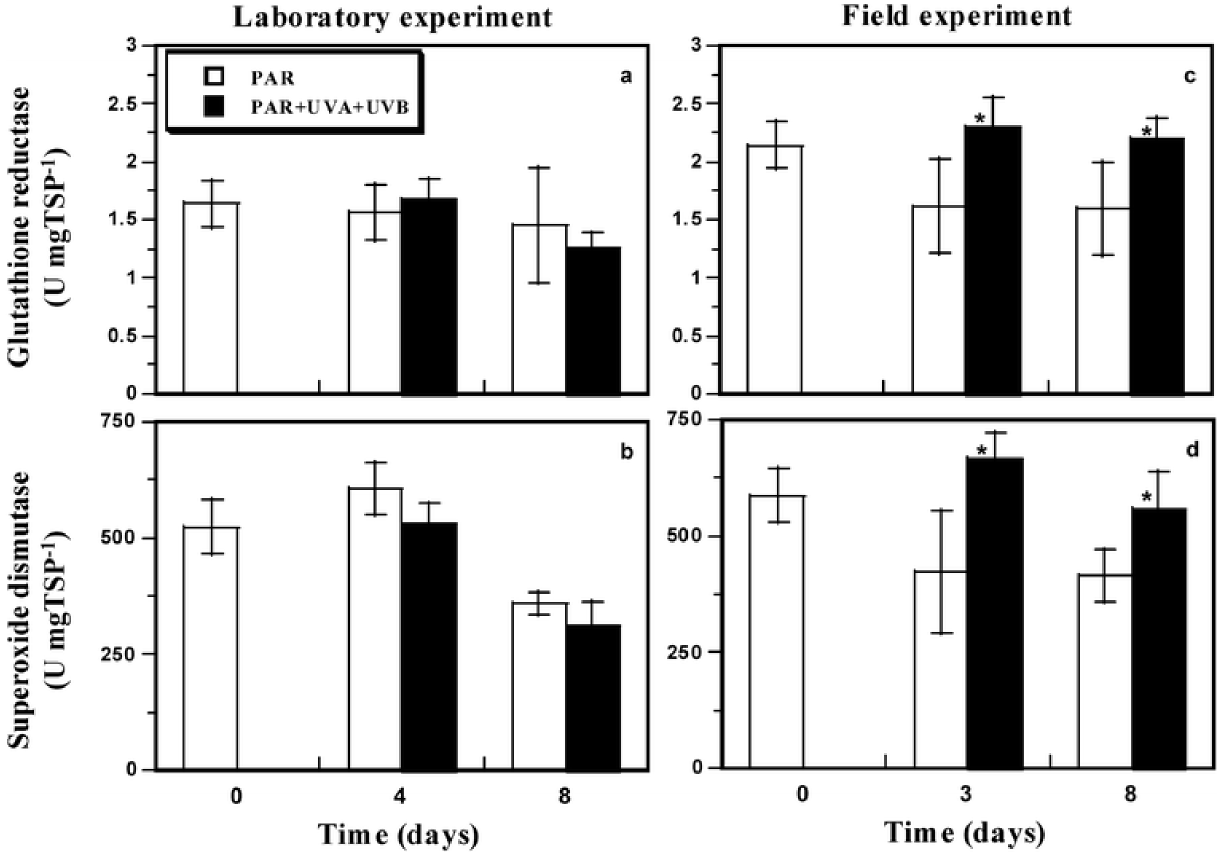

Fig. 3a-d. Changes of the activities of glutathione reductase (*GR*) and superoxide dismutase (*SOD*) in the Arctic green alga *Acrosiphonia penicilliformis*. **a**, **b** Laboratory experiment: continuous exposure under artificial PAR (*grey bars*) and PAR+UVA+UVB (*black bars*) radiation; **c**, **d** Field experiment: exposure under total (*black bars*) and UVA+UVB cut-off solar radiation by means of a selective UV cut-off filter (cut off at <395 nm.). Data are given as mean values±SD (*n*=3) and expressed as Units mg^{-1} total soluble proteins (*TSP*). Mean values with different *asterisks* are significantly different (at *P*=0.05) to the control

Discussion

The present study provides a survey of the qualitative and quantitative content and activities of different reactive-oxygen-scavenging enzymes in 22 macroalgal species from the Arctic. We detected a great variation with respect to the antioxidant enzyme activities and found strong species-specific differences. High activities of antioxidant enzymes found in green algae were comparable to those of higher plants and microalgae. For example, *M. arcticum*, which showed the highest activity in SOD within the investigated macroalgae, is in the same activity range as reported for pea leaves (Moran et al. *1994*). CAT activity of *M. arcticum* is half as high as that of cotton fibres (Rajguru et al. *1999*), whereas activities of SOD and GR were much higher. In comparison with other algae, APX activities of *M. arcticum* are comparable to those of the symbiotic zooxanthellae of the sea anemone, *Aiptasia pallida* (Lesser and Shick *1989*) while CAT and SOD activities are much higher. Data with respect to other macroalgae are scarce and only the data on the Arctic brown alga *Fucus distichus* (Table 2) can be directly compared to those of similar species from temperate water (Collen and Davison *1999a*). CAT activity of *F. distichus* was similar to that reported by other authors for the same species while SOD activity was much higher in the polar species and lower activities were measured for GR and

APX. The ascorbic acid concentrations found in green algae are very high, similar to those reported, in general, for lemons and oranges. *Chaetomorpha linum*, for example, contains 0.65 mg ascorbic acid g^{-1} FW, a concentration similar to that of lemon, with approximately 0.69 mg g^{-1} FW. *F. distichus* from Spitsbergen contained ascorbic acid values in the same range as in this and other *Fucus* species from temperate regions (Collen and Davison *1999a*).

A closer analysis points to the relation between antioxidant activities and depth distribution. In particular, differences in SOD activity between algal groups are related to the depth distribution on the shore. Most green algae, which showed higher antioxidant activities, typically inhabit the upper part of the shore at the Kongsfjord. Similarly, the red algae, *D. ramentacea* and *Palmaria palmata*, occurring frequently in the upper sublittoral, exhibited higher SOD activities compared to red-algal species living in deeper waters, such as *Phycodrys rubens*. Additionally, green algae contain relatively high amounts of ascorbic acid. Therefore, species more exposed to drastic and rapid changes in environmental radiation conditions have developed an efficient biochemical defence system to withstand the stress.

Although directly exposed to solar radiation, the supralittoral species *Prasiols crispa* growing underneath bird colonies exhibits very low antioxidant enzyme activities in comparison to the other green algae. In this plant from such an unusual habitat, it seems that another photoprotective strategy is developed, such as the biosynthesis of UV-absorbing compounds that are known to prevent radiative damage (Dunlap and Shick *1998*). In *Prasiola crispa* ssp. *antarctica* from Antarctica, high amounts of two new, so far chemically uncharacterized, mycosporine-like amino acids (MAAs) have been reported (Hoyer et al. *2001*). MAAs represent a group of compounds with a potential role as UV sunscreens, exhibiting absorption maxima between 310 and 360 nm (Karentz et al. *1991*). Their accumulation is positively correlated to the extent of UV exposure, as shown in laboratory and field studies (García-Pichel and Castenholz *1991*; Karsten et al. *1998*; Lesser *1996b*; Shick et al. *1995*). *Prasiola* is the only genus within the green algae containing MAAs. At present it is unknown whether the uncharacterized MAAs in *Prasiola crispa* also exhibit antioxidative properties as described for other MAAs (Dunlap and Yamamoto *1995*).

Compared to other algal taxa, antioxidant enzyme activities in brown algae are low. However, there is a strong adaptation and/or acclimation potential of photosynthesis (Bischof et al. *1998a*, *b*, *2000*) and growth (Aguilera et al. *1999a*) to UV radiation in this macroalgal group. In this context we refer to the typically high content of phenolic compounds in brown algae (Ragan and Glombitza *1986*; Van Alstyne and Paul *1990*), since these substances can act as antioxidants by transferring hydrogen atoms to lipid peroxyl radicals (Foti et al. *1994*). However, their role as antioxidants may be questionable because they are accumulated in special compartments, the physodes (Schoenwaelder *2001*), rather than uniformly distributed in the protoplasm. Another explanation for the high adaptation and acclimation potential of brown algae to UV radiation may be the ability of phenolic compounds such as phlorotannins to act as UV sunscreen pigments, as suggested by Pavia et al. (*1997*).

The investigation of the effects of the ultraviolet waveband of the solar spectrum on polar marine ecosystems has become an important ecological issue as a result of a gradual depletion of the ozone layer in both hemispheres. Exposed organisms have developed different strategies for protection against this biologically harmful radiation. However, almost no studies on the mechanisms of production of reactive oxygen species by UV radiation, and the biochemical defence strategies against this reactive species, have been performed for macroalgae. In order to analyse the ecophysiological importance of the total UV region (UVA+UVB) of solar radiation, laboratory and field experiments have been performed. Our study has clearly shown that the activity of antioxidant enzymes is stimulated by UV radiation in several Arctic macroalgae. The combination of artificial UVA+UVB radiation increased the GR activity in *M. arcticum*, *Coccotylus truncatus* and *Phycodrys rubens* after

84 h under continuous exposure. GR stimulation under UV radiation indicates an active scavenging of H_2O_2 by means of the ascorbate-glutathione cycle in combination with the Mehler-peroxidase reaction, which is the major pathway for scavenging potentially toxic intermediates of oxygen metabolism in photosynthesis, which at the same time enables down-regulation of electroflux (Polle *1996*). Dehydroascorbate formed by oxidation of ascorbic acid for scavenging of H_2O_2 by means of ascorbate peroxidase is reduced again to ascorbate, taking electrons from reduced glutathione by means of dehydroascorbate reductase. The product of these reactions, glutathione disulphide (GSSG), is reduced by the activity of GR and consumption of NADPH. Plants have been shown to increase GR activity in response to stress (Edwards et al. *1994*). Increments of GR activity in response to UV radiation have been described in *Arabidopsis* (Kubo et al. *1999*; Rao et al. *1996*). In Arctic macroalgae, it seemed to be a faster stimulation of GR activities after 24 h exposure, followed by incubation in darkness again for 24 h, indicating some kind of a dark-enhanced repair system after damage in light. Recovery from UV damage in low light or darkness has been extensively investigated in macroalgae, especially in studies on photoinhibition of photosynthesis (Hanelt *1996*, *1998*). Thus, stimulation of the biochemical system involved in the scavenging of reactive oxygen species generated in the photoinhibitory status, mediates this recovery in photosynthesis. The role of antioxidants in the partial recovery of photosynthetic performance has been studied in symbiotic cnidarians and their zooxanthellae (Lesser and Shick *1989*). According to these authors, the fluxes of reduced oxygen intermediates cause damage to the photosynthetic apparatus. In contrast to GR activities, no UV effects on SOD activities were found in *M. arcticum*, *Coccotylus truncatus* and *Phycodrys rubens* after this period of treatment. However, in *Palmaria palmata*, UV radiation seemed to directly affect the SOD activity and a decrease, just after the 1st day of culture, was observed in comparison to the PAR control. These results are comparable to those observed in the green microalga, *Chlorella vulgaris*, in which long-term effects of increasing UVB radiation resulted in a decrease in SOD activities (Malanga and Puntarulo *1997*). The reason for this negative effect may be an inhibition of gene expression for this enzyme as observed by Strid (*1993*) in *Pisum sativum* or an unspecific effect on enzyme activity. In contrast, Lesser and Shick (*1989*) found a stimulation of SOD activities in the symbiotic zooxanthellae of *Aiptasia pallida* by UV radiation. In that species, an increase in the SOD activities was correlated with an increase in the CAT activity, while in the present work no significant differences were found in the red algae *Palmaria palmata* and *D. ramentacea* after 8 days treatment.

No effects of artificial UV radiation on GR and SOD have been observed in the green alga, *Acrosiphonia penicilliformis*. In contrast, a significant reduction in GR and SOD activities was observed when natural UV radiation was cut off by selective filter foils in the field. This means that survival of this species in the intertidal zone is mediated by an enhanced oxygen-reactive scavenging system, in combination with morphological strategies, as reported by Aguilera et al. (*1999a*). While the apical region of this plant is mainly exposed to strong solar radiation, the basal cells are well protected due to self-shading. In the field, yellow-coloured tips were often observed, indicating lack of chlorophyll as a consequence of photobleaching of the apical parts, along with dark-green pigmented, healthy and unstressed basal parts.

In conclusion, different biochemical capabilities of the enzymatic defence systems against reactive-oxygen species were observed for several Arctic macroalgae of different taxa and habitats. The antioxidant enzyme activity is enhanced in species that grow in the upper part of the rocky shore, where they are exposed to drastic changes in environmental conditions, especially those related to rapid and drastic changes in the UV region of the solar spectrum.

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