pEffects of UV radiation on the ultrastructure of several red algae

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SUMMARY

The effect of ultraviolet (UV) radiation on the ultrastructure of four red algae, the endemic Antarctic *Palmaria decipiens* (Reinsch) Ricker and *Phycodrys austrogeorgica* Skottsberg, the Arctic-cold temperate *Palmaria palmata* (Linnaeus) O. Kuntze and the cosmopolitan *Bangia atropurpurea* (Roth) C. Agardh was studied. All four species showed a formation of 'inside-out' vesicles from the chloroplast thylakoids upon exposure to artificial UV-radiation. In *P. decipiens*, most vesicles were developed after 8 h and in *P. palmata*, after 48 h of UV exposure. In *B. atropurpurea*, vesiculation of thylakoids was observed after 72 h of UV irradiation. In *Ph. austrogeorgica*, the chloroplast envelope and thylakoid membranes were damaged and the phycobilisomes became detached from the thylakoids after 12 h of UV exposure. Ultraviolet-induced changes in the membrane structure of mitochondria were observed in *P. decipiens* and *P. palmata*. However, in *P. decipiens* they were reversible as was the damage in chloroplast fine structure after 12 h of UV treatment. Protein crystals in *Ph. austrogeorgica* showed degradation after exposure to UV radiation. Different methods of fixation and embedding macroalgal material are discussed. These findings give insight into the fine structural changes which occur during and after UV exposure and indicate a relationship between the species dependent sensitivity to UV-exposure and the depth distribution of the different species.

Key words: macroalgae, protein crystals, Rhodophyta, ultrastructure, UV radiation.

INTRODUCTION

Since 1979, a marked depletion of the stratospheric ozone layer, especially over Antarctica, has been observed during the early Austral summer leading to an increase in ultraviolet-B (UV-B) radiation at the Earth's surface (Frederick and Snell 1988; Karentz 1994). Although UV-B radiation is more strongly absorbed than photosynthetically active radiation (PAR) even in clear Antarctic waters, biologically relevant irradiances of UV-B radiation may penetrate the water column to 10–30 m at depth (Karentz 1989; Hoyer *et al*. 2001). Therefore, increasing solar UV radiation may be harmful, especially to polar and eulittoral species, which become fully exposed to natural sunlight during low tide (Holm-Hansen *et al*. 1993).

Ultraviolet radiation has been found to affect marine macroalgae in several ways, including effects on photosynthesis, DNA lesions, nitrogen metabolism and growth, as shown in numerous publications reviewed by Franklin and Forster (1997) and Bischof *et al*. (2002). Laboratory investigations have revealed multiple molecular targets of UV radiation, including nucleic acids (Harm 1980), photosynthetically relevant proteins (Vass 1997) and membrane lipids (Murphy 1983). The potentially most vulnerable lipids are those with unsaturated fatty acids that can easily be peroxidized by the action of oxygen radicals produced by UV irradiation impairing membrane stability (Kramer *et al*. 1991).

While the effects of UV radiation on the physiology of algae are relatively well documented, little is known about the influence on the cellular fine structure. Apart from a few investigations on higher plants and microalgae, there has only been one paper published dealing with UV-induced changes in the ultrastructure of the Antarctic red alga *Palmaria decipiens* (Reinsch) Ricker (Poppe *et al*. 2002). The results obtained by Poppe *et al*. (2002) are discussed more broadly in the present study and compared with studies of three other species.

MATERIALS AND METHODS

Plant material

Species used in this experiment are listed in Table 1. After collection, the isolates were kept in stock cultures at the Alfred Wegener Institute (Bremerhaven, Germany). Experimental individuals were raised from stock cultures at 5°C under various light conditions summarized in Table 2. Photosynthetically active radiation (PAR) was provided by daylight-fluorescent tubes (Osram

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Table 1. Investigated species and respective vertical distribution

Species	Class	Collecting location	Zonation (reference)
Palmaria decipiens (Reinsch) Ricker	Florideophyceae	King-George-Island (Antarctica)	upper/middle littoral (Wiencke and Clayton 2002)
Palmaria palmata (Linnaeus) O. Kuntze	Florideophyceae	Roscoff (France)	littoral down to 20 m (Bird and McLachlan 1992)
Phycodrys austrogeorgica Skottsberg	Florideophyceae	King-George-Island (Antarctica)	littoral down to 43 m (Wiencke and Clayton 2002)
Bangia atropurpurea (Roth) C. Agardh	Bangiophyceae	Disko-Island (Greenland)	upper littoral down to 8 m (Bird and McLachlan 1992)

Table 2. Exposure conditions

Species	Preculture and recovery light conditions (μmol photons m^{-2} s^{-1})	UV exposure (h)	PAR exposure after UV exposure (h)
Palmaria decipiens	25	23	24
Palmaria palmata	25	48	48
Phycodrys austrogeorgica	10	12	24
Bangia atropurpurea	40	96	48

L58/W19, Osram, Berlin, Germany) under 18 h : 6 h light : dark cycles. The cultures were aerated in glass beakers filled with filtered sea water (0.2 μm membrane filters, Sartorius Sartobran, Germany) which was changed every 10 days. The medium was enriched with nutrients after Provasoli as described in Stein (1973).

UV irradiation conditions

In the experimental treatment, samples were exposed to artificial UV radiation supplied by UVA-340 fluorescent tubes (Q-Panel, Cleveland, USA). Background PAR emitted by additional daylight fluorescent tubes (Osram L58/W19) was adjusted to the respective irradiance in the precultures (see Table 2). Spectral irradiance conditions within the experimental set-ups using different UV irradiances were determined with a Spectro 320 D spectroradiometer (Instrument Systems, Munich, Germany) (see Fig. 1). The integrated irradiances of PAR (400–700 nm), UV-A (320–400 nm) and UV-B (280–320 nm) are summarized in Fig. 1. After the UV treatments, the algae were transferred again to the respective preculture conditions to induce recovery (see Table 2).

Electron microscopy

Samples were cryofixed by use of a high-pressure freezing machine (HPM 010, Balzers, Liechtenstein) as described by Dahl and Staehelin (1989). Freeze-substitution was performed in an automatic freeze-substitution system (AFS, Reichert, Austria) using two different substitution protocols. In fixation 1, a mixture of dimethoxypropan (DMP) : water free acetone (1 : 2 v/v) with 2% glutaraldehyde (from a 70% stock solution),

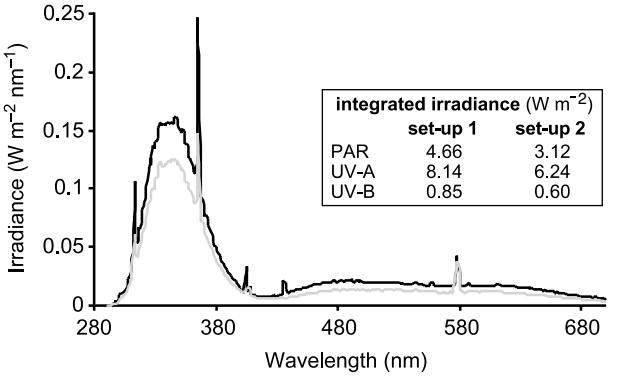
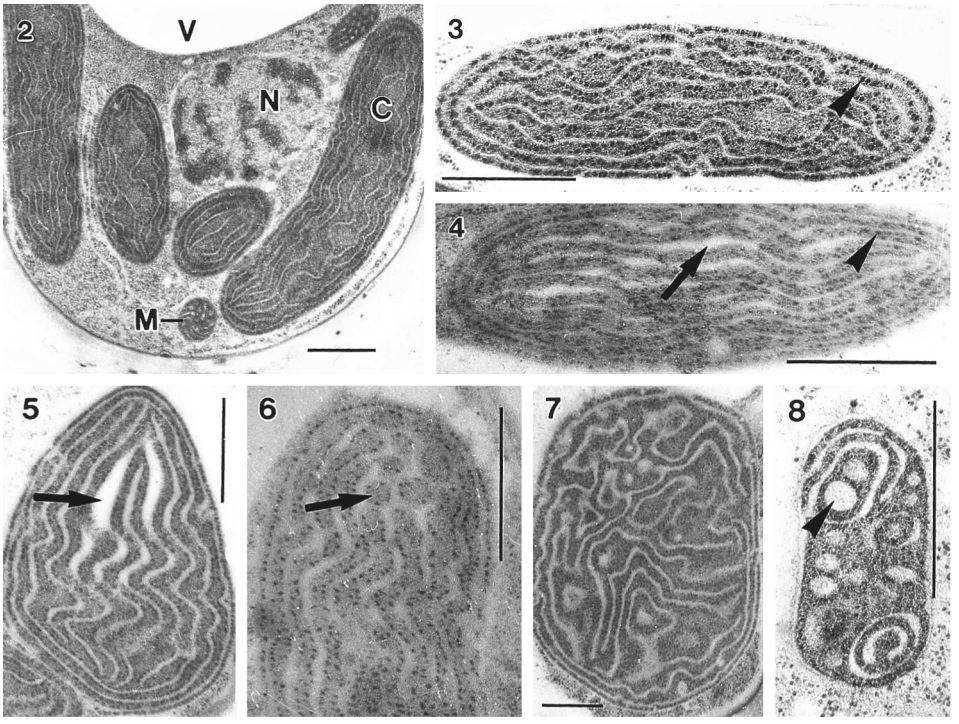


Fig. 1. Spectral irradiance of the UV-range (280–400 nm) and PAR (400–700 nm) emitted by the lamps used in the experiments. (———), set-up 1; (———), set-up 2.

0.6% uranyl acetate (from a 10% stock solution in water-free methanol) and 1.5% osmium tetroxide was used as described by Kaeser (1989). Samples were kept in the medium at –75°C for 72 h, warmed from –75°C to –60°C over 3 h, held at –60°C for 72 h, warmed from –60°C to –30°C over 6 h, held at –30°C for 24 h. Samples were then washed in DMP : acetone (1 : 2 v/v) for 1 h and in water-free acetone for 1 h and finally warmed to room temperature. In fixation 2, a parallel set of samples was freeze-substituted according to the protocol of Steinbrecht and Müller (1987). Samples were kept in water-free acetone with 0.5% uranyl acetate at –90°C for 50 h, warmed from –90°C to –60°C over 2 h, held at –60°C in 2% osmium tetroxide in dried acetone for 10 h, warmed from –60°C to –30°C in dried acetone for 2 h, held at –30°C for 10 h and finally warmed to room temperature. Samples were then infiltrated gradually in Araldite



Figs 2–8. Effect of ultraviolet (UV) radiation on the fine structure of *P. decipiens*. Scale bars = 1 μm. 2. Fine structure from an UV-untreated specimen with typical chloroplasts and mitochondria (fixation 2). 3. Typical chloroplast fine structure in a UV-untreated individual (fixation 2). Arrowhead points to a phycobilisome. 4. Chloroplast fine structure in a specimen irradiated with UV for 2 h (set-up 2), showing dilated thylakoids (arrow). Arrowhead points to a phycobilisome. 5. Chloroplast fine structure in a specimen irradiated with UV for 4 h (set-up 2), showing dilated thylakoids (arrow). 6. Chloroplast of a specimen irradiated with UV for 6 h (set-up 1) showing formation of 'inside-out' vesicles (arrow) (fixation 1). 7. Chloroplast of a specimen irradiated with UV for 23 h (set-up 2) showing disorganized thylakoid formation (fixation 2). 8. Mitochondrion of a specimen irradiated with UV for 8 h (set-up 2), with cristae enlarged to sacculi (arrowhead) (fixation 2). C, Chloroplast; M, Mitochondrion; N, Nucleus; V, Vacuole.

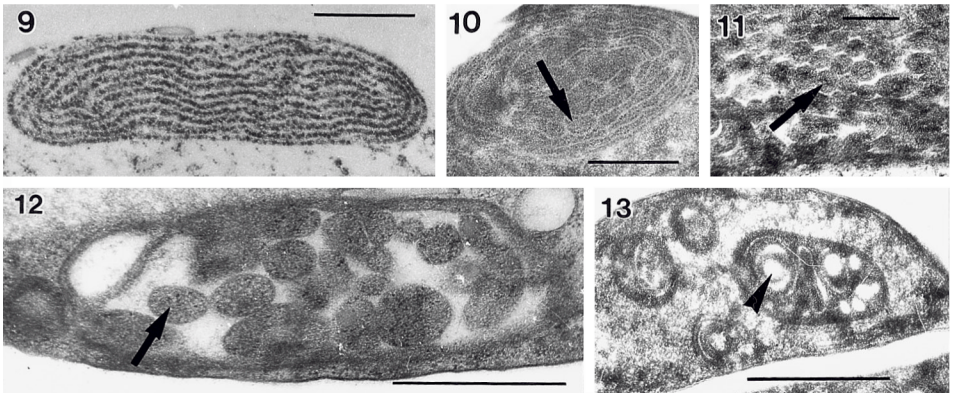
(fixation 1) (Plano, Germany) or in Spurr's low-viscosity resin (fixation 2) (Spurr 1969) (Plano, Germany) and polymerized 7 days later at 70°C. Sections were cut on an ultramicrotome (ULTRACUT E, Reichert, Austria) using a diamond knife, collected on formvar-carbon coated copper grids and stained with uranyl acetate and Reynolds' lead citrate (Reynolds 1963). The samples were examined in an electron microscope (EM 902, Zeiss, Germany) at 80 kV.

RESULTS

Palmaria decipiens

UV-untreated cells showed the typical fine structure of florideophyceaen cells. The chloroplasts in particular, exhibited the normal pattern of parallel thylakoids and phycobilisomes attached to the outside of the thylakoid membranes (Figs 2,3). In cells from algae irradiated with UV-A + B radiation for 2 h, chloroplast thylakoids appeared dilated (Fig. 4). Moreover, vesicle-like formations at the margin of chloroplasts were observed. After 4 h of UV radiation, these changes were more pronounced. Figure 5 shows a chloroplast with highly dilated thylakoids. Chloroplasts with vesicle-like appearances were found very rarely. After 6–8 h of UV radiation, pronounced changes in the ultrastructure of chloroplasts were observed (Fig. 6). The chloroplast thylakoids were disrupted and appeared to be connected with vesicles of up to 0.5 μm in diameter. Stroma and phycobilisomes can still clearly be recognized inside the vesicles. These structural changes were predominantly present on the side of the thallus exposed to the UV source, whereas the shaded side was less affected. After 12–23 h of UV exposure, these changes disappeared. No additional vesicles were observed and the phycobilisomes were attached to the protoplasmic surface of the thylakoids. However, the thylakoids exhibited an irregular shape (Fig. 7).

The mitochondria of UV-untreated individuals exhibited a tubuli-type structure (Fig. 2). After 4 h of UV exposure however, the tubuli were swollen and appeared mostly as sacculi (Fig. 8). These changes disappeared after 23 h of UV irradiation. Nuclei, Golgi bodies and endoplasmic reticulum (ER) did not show any conspicuous structural changes upon UV exposure.



Figs 9–13. Effect of ultraviolet (UV) radiation on the fine structure of *P. palmata*. Scale bars = 1 μm. 9. Typical chloroplast fine structure in a UV-untreated individual (fixation 1). 10. Chloroplast of a specimen irradiated with UV for 2 h (set-up 1), showing vesiculation of thylakoids (arrow) (fixation 1). 11. Part of a chloroplast from a specimen irradiated with UV for 24 h (set-up 1), showing tubular-like structures formed out of thylakoids (arrow). 12. Chloroplast of a specimen irradiated with UV for 48 h (set-up 1) and subsequent 48 h of low PAR, showing vesiculation of thylakoids (arrow) and highly expanded intrathylakoidal spaces (fixation 1). 13. Mitochondrion of a specimen irradiated with UV for 48 h (set-up 1), with cristae enlarged to sacculi (arrowhead) (fixation 1).

Palmaria palmata

The fine structure of cells from UV-untreated individuals from *P. palmata* resembled that of *P. decipiens* with typical chloroplasts (Fig. 9) and rod-shaped mitochondria with tubular cristae. The first changes in the structure of chloroplasts were detectable after 2 h of UV exposure. Chloroplasts showed a vesiculation of thylakoids (Fig. 10). After 6 h of UV radiation, the vesiculation of the thylakoids increased; vesicles or tubular structures were regularly organized. These changes persisted until 24 h of UV exposure (Fig. 11). After 48 h of UV exposure, these vesicles grew in size and the electron translucent intrathylakoidal space expanded. Under subsequent exposure to white light for another 48 h this tendency was even more strongly pronounced (Fig. 12).

Mitochondria of UV-untreated cells exhibited a tubuli-type structure (data not shown). After 2 h of UV exposure, they appeared to be swollen and the inner mitochondrial membrane was organized in sacculi. These changes are still detectable after 48 h of UV radiation and even after 48 h of exposure to white light only (Fig. 13).

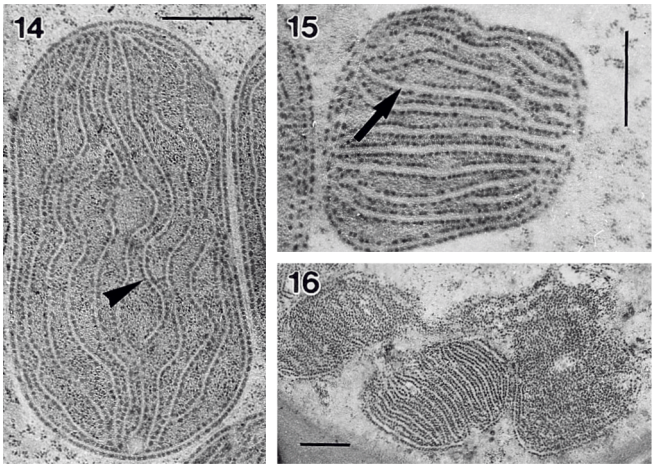
Phycodrys austrogeorgica

Chloroplasts from UV-untreated individuals from *Ph. austrogeorgica* show the fine structure characteristic for the Florideophyceae with a high number of chloroplasts. The chloroplasts are well preserved with the normal pattern of thylakoids arranged in parallel and phycobilisomes attached to the thylakoid membranes (Fig. 14). After 2–7 h of UV exposure, some chloroplasts showed dilated thylakoids (Fig. 15). A vesiculation of thylakoids with stroma and phycobilisomes inside was detectable occasionally in samples irradiated for 6 h with UV. After 8 h of UV radiation, a disorganized pattern of thylakoid arrangement could be observed and after 12 h of exposure, there was a visible breakdown of the chloroplast membrane structures. Specifically, phycobilisomes became detached from the thylakoid membranes, and the thylakoids and chloroplast envelope disintegrated (Fig. 16). After 24 h of exposure to white light for recovery, the chloroplasts still appeared damaged (data not shown).

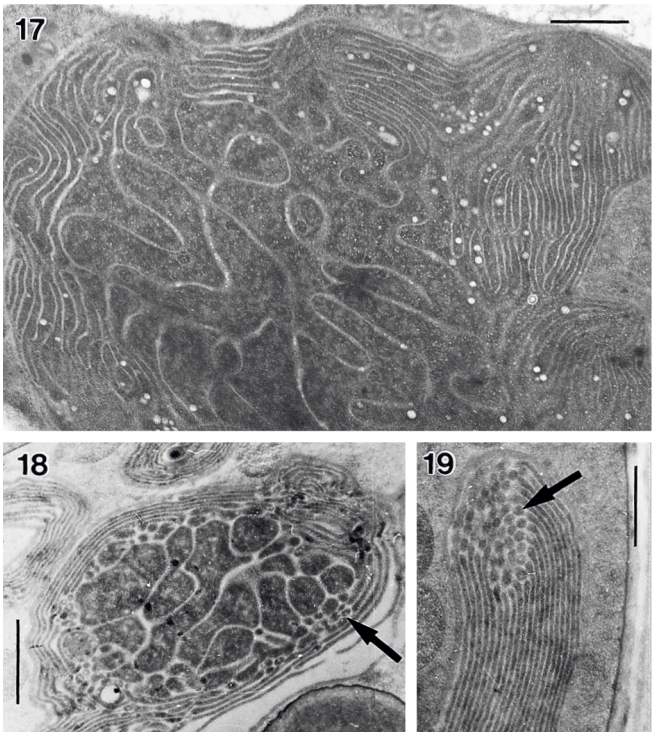
Ph. austrogeorgica shows a variety of crystalline, electron-dense inclusions in the cytoplasm. One type consists of a crystalline substructure with no visible surrounding membrane (Fig. 20). After 4 h of UV exposure, these inclusions had a lamellar structure with discontinuities in the crystal matrix (Fig. 21). After 12 h of UV radiation there was a loosening of the lamellar substructure at the periphery of the crystal, as well as internal fracturing (Fig. 22). After re-exposure to PAR, the crystal became further degraded (Fig. 23). Another type of inclusion in the *Ph. austrogeorgica* cytoplasm is characterized by a highly electron-dense material with no visible crystalline substructure (Fig. 24). Under UV exposure, the crystals with a diameter of *c.* 2 μm break up into several smaller crystals with less electron-dense material around the dark areas (Fig. 25).

Bangia atropurpurea

Control specimen from *B. atropurpurea* showed the typical stellate-shaped chloroplast characteristic for the Bangiophyceae with thylakoids organized in parallel and a central pyrenoid (Fig. 17). After 72 h of UV exposure, this pattern changed into vesicle-like appearances adjacent to the pyrenoid (Fig. 18) and tubularly arranged thylakoids (Fig. 19). In severe cases, these structures grew in size and vesicles of 0.5 μm in diameter were formed (Fig. 18).



Figs 14–16. Effect of ultraviolet (UV) radiation on the fine structure of *Ph. austrogeorgica*. Scale bars = 1 μm. 14. Typical chloroplast fine structure in a UV-untreated individual (fixation 2). Arrowhead points to a phycobilisome. 15. Fine structure of a chloroplast in a specimen, irradiated with UV for 4 h (set-up 2), showing dilated thylakoids (arrow) (fixation 2). 16. Chloroplasts from a specimen irradiated with UV for 12 h (set-up 2), showing detached phycobilisomes and a damaged chloroplast envelope (fixation 2).

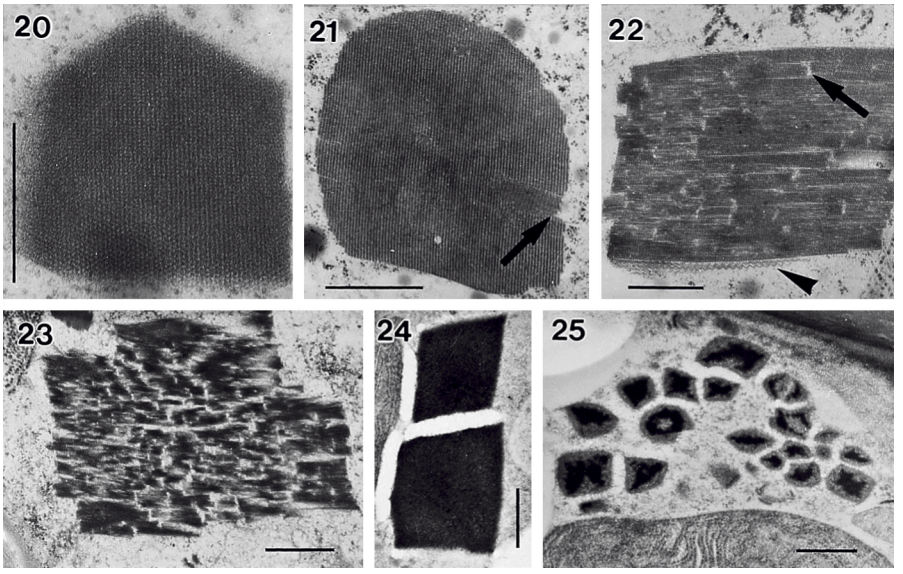


Figs 17–19. Effect of ultraviolet (UV) radiation on the fine structure of *B. atropurpurea*. Scale bars = 1 μm. 17. Chloroplast fine structure in a UV-untreated individual (fixation 1). 18. Chloroplast in a specimen irradiated with UV for 72 h (set-up 1), showing vesicles formed out of thylakoids adjacent to the pyrenoid (arrow) (fixation 1). 19. Chloroplast in a specimen irradiated with UV for 72 h (set-up 1), showing tubular thylakoids (arrow) (fixation 1).

DISCUSSION

Methodological considerations

The present study showed that cryofixation followed by freeze-substitution is a reliable method to preserve cellular fine structure and phycobilisomes in particular, in red macroalgae. The high pressure freezing method for fixation of subcellular structures with subsequent freeze-substitution represents the best method to show intact phycobilisomes in red macroalgae, which is a major advantage compared to conventional chemical fixation. Up to now, intact phycobilisomes attached to the thylakoid membrane have been mainly shown in cyanobacteria and unicellular red algae (Gantt and Conti 1967; Van Eykelenburg 1979; Mörschel and Rhiel 1987; Lange *et al*. 1990), they have not been



Figs 20–25. Effect of UV radiation on protein crystals of *Ph. austrogeorgica*. Scale bars = 1 μm or as indicated. 20. Protein crystal in an untreated individual showing periodicity of the crystal (fixation 2). Scale bar = 0.5 μm. 21. Protein crystal in a specimen irradiated with UV for 4 h (set-up 2), showing a lamellar substructure and discontinuities in the crystal matrix (arrow) (fixation 2). 22. Protein crystal in a specimen irradiated with UV for 12 h (set-up 2), showing loosening of the lamellar substructure on the edge of the crystal (arrowhead) and a break-up of the whole structure (arrow) (fixation 2). 23. Protein crystal in a specimen irradiated with UV for 12 h (set-up 2) and subsequent recovery in preculture white light conditions for 24 h, showing further degradation of the whole crystal (fixation 2). 24. Protein crystals in a UV-untreated specimen characterized by a highly electron-dense material with no visible crystalline substructure (fixation 1). 25. Protein crystals in a specimen irradiated with UV for 6 h (set-up 1), showing several electron-dense subcrystals in a matrix of more electron-transparent material (fixation 1).

shown well in macroalgae (Waaland *et al*. 1974; Pueschel and van der Meer 1984; Rascio *et al*. 1991; Foltran *et al*. 1996; Talarico 1996; Tsekos *et al*. 1996). In only one other study have the phycobilisomes in the Antarctic red alga *Palmaria decipiens* been well preserved by the use of cryofixation, as in the present study (Poppe *et al*. 2002). Chemical fixation depends on the infiltration time of the fixative, therefore cryofixed biological samples show the native state rather than chemically fixed ones. In many cells, the best preparation for the preservation of cellular ultrastructure is high pressure freezing (Gilkey and Staehelin 1986; Kiss and Staehelin 1995). However, cryofixation followed by freeze-substitution also has its limitations, for example a reduced infiltration of resin and poor membrane contrast (Babuka and Pueschel 1998). In the present study, the problems of poor membrane contrast in cryofixed specimen is evident, although this feature appears to be resin-dependent. Chloroplasts of specimens embedded in Araldite (fixation 1) show a good preservation of phycobilisomes but low membrane contrast, while chloroplasts of specimens embedded in Spurr's resin (fixation 2) or Epon 802 (data not shown) show a slightly better membrane contrast.

UV effects on ultrastructure

The most striking effect of UV radiation on the fine structure of red algae in this study is the disruption of the chloroplasts. The formation of 'inside-out' vesicles from the thylakoid membrane occurred in all four species examined and therefore may appear as a general rule for red algal chloroplasts. Moreover, in the extremely sensitive *Ph. austrogeorgica* protein crystals appear corroded under UV radiation indicating either damage or remobilization of the stored protein for repair processes.

From this study, it appears that the chloroplasts are sensitive to UV radiation in red algae. Disruption of thylakoids and the formation of translucent 'inside-out' vesicles with phycobilisomes inside have been previously documented in *Palmaria decipiens* by Poppe *et al*. (2002). Similar vesicles were observed in the pea *Pisum sativum* L. (higher plant) and are thought to be formed from thylakoids after exposure to UV-B-radiation (Brandle *et al*. 1977). Complete disruption of the outer double membranes of the chloroplasts was also documented. A general dilation of thylakoids after irradiation of *Beta vulgaris* L. (higher plant) leaves with low UV-B irradiances (290–320 nm, 94.6 mW m^{-2}) was reported by Bornmann *et al*. (1983). However, 'inside-out' vesicles were not reported in this species. A disintegration of the grana and a loss of the net-like arrangement was observed in UV-irradiated cells of the unicellular green alga *Micrasterias denticulata* Breb. under unnaturally high UV-B irradiances (UV-B 9 W/m^2, UV-A 26.8 W/m^2) (Meindl and Lütz 1996; Lütz *et al*. 1997). It should, however, be mentioned that in

the *Micrasterias* studies a non-natural UV-A:UV-B ratio of 3 : 1 was applied, which differs significantly from the natural ratios observed in the field of about 20 : 1. The UV-B irradiance applied in the present study (0.6 W/m^2) corresponds more closely to the field conditions observed in Antarctica during summer (Hoyer *et al*. 2001).

The fine structural changes shown in the present study, especially the formation of the 'inside-out' vesicles may have functional implications. Physiologically, it would be difficult to establish a proton gradient across the thylakoid membrane and consequently, photosynthetic reactions will be impaired. Other reported UV-induced changes include depolarization of the membrane potential, net leakage of Cl^-, Na^+ and K^+ and differences in ATPase activity in cell membranes (Doughty and Hope 1973; Murphy 1983; Gallo *et al*. 1989) and in thylakoid membranes (Iwanzik *et al*. 1983; Chow *et al*. 1992; Hideg and Vass 1996). These changes in ion-permeability of the thylakoid membranes take place within a few hours of UV-B exposure, and should therefore be taken into account when considering the results of the present study. Furthermore, it has been found that UV radiation leads to a depletion of unsaturated fatty acids in membranes through lipid peroxidation, therefore decreasing the unsaturated/saturated fatty acid ratio and impairing membrane stability (Kramer *et al*. 1991). In the worst cases, whole chloroplasts are disrupted as shown in the present study of *Ph. austrogeorgica*. This high level of damage reflects the extreme sensitivity of the chloroplasts of this sublittoral species, fitting well with studies on photosynthetic performance after UV exposure in the same species (Bischof *et al*. 1998). In contrast to *P. decipiens*, maximum quantum yield in *Ph. austrogeorgica* is not able to recover in 24 h white light after exposure to 12 h of UV stress (data not shown). The extreme UV sensitivity of this species may be related to the fact that *Ph. austrogeorgica* has no capability for biosynthesis of sun-screens such as mycosporine-like amino acids (MAA) (Hoyer *et al*. 2001) which have been shown to be important as UV-absorbing compounds in red algae (Karentz *et al*. 1991). Under the low UV levels in the sublittoral, this species is able to survive and reproduce. However, if UV levels increase, this study suggests that the survival of this species at these depths is not possible.

Ph. austrogeorgica is regarded as the species most sensitive to UV radiation in the present study. The other three species, all growing in more exposed habitats, react to UV radiation with strong vesiculation in the chloroplasts. However, they are less sensitive to UV radiation than *Ph. austrogeorgica* and do not show a total breakdown of chloroplast fine structure or a leakage of phycobilisomes. One reason for the difference in sensitivity may be the high concentrations of MAA in both *Palmaria* species (Karsten *et al*. 1998; Karsten and Wiencke 1999; Hoyer *et al*. 2001) and the even higher MAA concentration in *Bangia atropurpurea* (Karsten and West 2000).

The reformation of an almost normal thylakoid structure as shown in *P. decipiens* seems to indicate an acclimation process to UV radiation. This interpretation is supported by the fact that after 16 h of UV treatment, maximum quantum yield was restored when *P. decipiens* was cultivated for 24 h in white light (Poppe *et al*. 2002). In *P. palmata* however, thylakoid vesicles did not disappear after 48 h UV exposure, indicating that this species has a higher sensitivity to UV radiation. However, in comparison with the deep-water species *Ph. austrogeorgica*, *P. palmata* is still more resistant to UV as there was no detachment of phycobilisomes seen in *Palmaria* species from shallow waters.

Ultraviolet-induced changes in the structure of mitochondria similar to those observed in *P. decipiens* and *P. palmata* in the present study (changes from tubuli to sacculi type structure), have also been documented in higher plants (Lichtscheidl-Schultz 1985; Santos *et al*. 1993). Physiologically, these findings may have functional implications, although Aguilera *et al*. (1999) showed a low sensitivity of respiration to UV radiation in various species including *Palmaria palmata*.

Another fine structural aspect shown here is the sensitivity of protein crystals to UV radiation. Protein crystals are protein storage pools which are mobilized under nitrogen limitations (Pueschel 1992). The present study shows for the first time that two types of protein crystals in *Ph. austrogeorgica* are either damaged by UV radiation, or that the protein is remobilized and used for repair processes. Proteins are known to be strong absorbers of UV-B radiation (Karentz 1994) and an increased protein degradation followed by resynthesis in order to replace UV-B sensitive proteins could thus be expected during UV-B exposure (Cullen and Neale 1994). Repair-mechanisms for UV-B induced damage of membranes or electron transport components demand increased enzymatic activity with higher nitrogen requirements. For example, photosynthetically relevant proteins like ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) or D1 protein show an increased turnover under UV exposure, leading to a decrease in photosynthetic activity (Aro *et al*. 1993; Bornmann and Teramura 1993; Strid *et al*. 1994). Moreover, it has been shown that UV-B can directly affect the nitrogen uptake system in phytoplankton and leads to decreased uptake rates of ammonium and nitrate (Döhler 1992; Behrenfeld *et al*. 1995). Therefore, one function of protein crystals may be the storage of nitrogen and this may provide a buffer against the limiting factor of a UV-induced nitrogen deficit.

The present study on ultrastructural changes confirms the correlation between the species dependent

sensitivity towards UV-exposure and the respective vertical distribution of species on shore. Further ozone depletion and the related increase in UV radiation on the Earth's surface is regarded as a threat to macroalgae growing in the deeper sublittoral, as these species lack protection from harmful UV radiation.

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