J Appl Phycol (2008) 20:387–395
DOI 10.1007/s10811-007-9268-2

UV-absorbing compounds in *Porphyra haitanensis* (Rhodophyta) with special reference to effects of desiccation

Hongxia Jiang · Kunshan Gao · E. Walter Helbling

Received: 23 June 2007 / Revised and Accepted: 19 September 2007 / Published online: 30 October 2007

Abstract The intertidal red alga *Porphyra haitanensis* Chang et Zheng is episodically desiccated and exposed to high levels of solar radiation at low tide during emersion. However, little has been documented on the relationship between the stresses during desiccation and related chemical compounds. We found that *P. haitanensis* thalli, when desiccated under indoor (artificial radiation) or outdoor (solar radiation) conditions, with or without UV radiation (UVR: 280–400 nm), contained significantly higher concentrations of UV-absorbing compounds (peak at 336 nm) than those maintained submerged (without desiccation). Solar UVR had no effect on the content of UV-absorbing compounds. Even though the concentration of these compounds decreased with time in all treatments, a slower decrease was observed in the desiccated samples. The samples with higher levels of UV-absorbing compounds showed higher photochemical efficiency of photosystem II (PS II) during the exposure or subsequent recovering process than samples with low concentration of UV-absorbing compounds, reflecting their protective role. The concentration of these compounds varied in different parts of the thallus, with the middle and marginal parts containing 60–80% more UV-absorbing compounds than the basal parts in both female and male plants. In addition, the marginal parts of male thalli contained more UV-absorbing compounds than the corresponding parts of female thalli. Our data suggest that desiccation plays a key role in this alga to maintain high concentration of UV-absorbing compounds, and that this might provide a beneficial advantage to compete in the intertidal zone where the organism is normally exposed to high levels of UVR.

Keywords Chemical compounds · Photochemical efficiency · *Porphyra* · UVR

H. Jiang · K. Gao (✉)
Marine Biology Institute, Shantou University,
Shantou, Guangdong 515063, China
e-mail: ksgao@stu.edu.cn

H. Jiang
Department of Biology and Food Technology,
Changshu Institute of Technology,
Changshu, Jiangsu 215500, China

K. Gao
State Key Laboratory of Marine Environmental Science,
Xiamen University,
Xiamen, Fujian 361005, China

E. W. Helbling
Estación de Fotobiología Playa Unión and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),
Casilla de Correos N(15-(9103),
Rawson, Chubut, Argentina

Introduction

Macroalgae are important as major primary producers in coastal regions, and many of them are also commercially farmed for food production or natural products. Most species of macroalgae are distributed in the intertidal zone, experiencing high levels of solar radiation. Ultraviolet radiation (UVR: 280–400 nm) has been found to cause a wide range of negative effects on macroalgae, such as photoinhibition (Franklin and Forster 1997; Han et al. 2003), DNA damage (Pakker et al. 2000; van de Poll et al. 2002), degradation of pigments (Aguilera et al. 1999), and reduction of spore germination (Han et al. 2004). However, solar UVR was also found to be less damaging to photochemical efficiency of some macroalgal species when given alone than when combined with high photosynthet-

ically active radiation (PAR: 400–700 nm) (Dring et al. 2001), and to enhance photosynthetic carbon fixation at low levels of solar irradiances (Helbling et al. 2003). Adaptation to UVR levels has equipped macroalgae with defensive mechanisms to minimize UV-induced damage. In addition to repair of UV-induced DNA damage by photoreactivation and nucleotide excision repair (Buma et al. 1995; Pakker et al. 2000; Lud et al. 2001a), an important mechanism to reduce the damaging impact of UVR in marine macroalgae is the synthesis and accumulation of UV-absorbing compounds. These compounds, mainly mycosporine-like amino acids (MAAs), screen off or reduce harmful radiation within the UV wavebands (Karsten et al. 1998).

As the main members of UV-absorbing compounds, MAAs are water soluble substances with absorption maxima ranging from 310 to 360 nm (Nakamura et al. 1982). Although their UVR-protective function is not yet completely clear, the most acceptable interpretation is that they play a role as a screen against UVR (Conde et al. 2000; Karsten et al. 2005). Some of these compounds may also function as antioxidants (Dunlap and Yamamoto 1995; Suh et al. 2003; Han and Han 2005) or as osmosis-regulating substances (Oren 1997). Accumulation of UV-absorbing compounds could be induced by different radiation treatments (Karsten et al. 1999, 2005; Korbee Peinado et al. 2004; Han and Han 2005) and nutrient availability (Korbee Peinado et al. 2004; Korbee et al. 2005), or affected by osmotic stress and other environmental stresses (Oren 1997; Klisch et al. 2002; Oren and Gunde-Cimerman 2007). However, little is known about the effect of desiccation on the UV-absorbing compounds concentration of intertidal species that are frequently exposed and desiccated during low tides, although it has been considered in other studies conducted with bryophytes, grass and lichens (Oliver et al. 2000; Lud et al. 2001b) as well as cyanobacteria (Fleming and Castenholz 2007). *Porphyra* spp. contain high levels of these compounds, mainly porphyra-334, and accumulate the highest concentrations among the species of red algae studied so far (Gröniger et al. 1999; Hoyer et al. 2001). UV-absorbing compounds concentration in *Porphyra columbina* was enhanced when exposed to UVR under high nitrogen levels (Korbee Peinado et al. 2004), and decreased steadily during the day under both photosynthetically active radiation (PAR) and UVR (Helbling et al. 2004). In *P. umbilicalis*, induction of these compounds by UVR was not found (Gröniger et al. 1999).

Porphyra haitanensis is endemic to the southern part of China and is commercially cultivated. In its natural habitats, it grows in the eulittoral zone, experiencing high solar irradiance and repeated desiccation during low tides. *P. haitanensis* shows enhanced photosynthesis with elevated CO_2 in air and utilizesHCO_3^- while submerged in water (Zou and Gao 2002a, b, 2004). However, little is known about the impact of solar UVR on this species. During the desiccation process, *P. haitanensis* experiences higher levels of UVR than when submerged. It is of general interest to know whether UV-absorbing compounds in this species would be affected by desiccation or UVR during low tide. The present study investigated the effects of desiccation and UVR on the concentration of UV-absorbing compounds in *P. haitanensis*.

Materials and methods

Thalli of *Porphyra haitanensis* Chang et Zheng were collected from the farmed areas around Nan'ao Island (117.09°E, 23.47°N), Shantou, China, during December, 2004 and November–December, 2005. Samples (both immature and mature thalli) were transported to the laboratory, and maintained in filtered aerated seawater at room temperature (15–20°C) and ≤50 μmol photons m^{-2} s^{-1} of PAR before being used for the experiments 1 day after collection. The mature thalli consisted of reproductive cells (sphere-shaped, in packets) in the marginal parts, vegetative cells (polygon-shaped) in the middle parts, and rhizoidal cells (balloon-shaped) in the basal parts. The immature thalli had only vegetative and rhizoidal cells. Mature and immature thalli were separated under a stereomicroscope so that the same type of thalli was used in each experiment. Before experimentation, the thalli were placed in glass containers or quartz tubes with filtered (Whatman GF/F) and sterilized seawater enriched with F medium (Guillard and Ryther 1962). Half of the volume of the enriched seawater in each culture was renewed every day. All cultures were aerated at a flow rate of about 0.4–0.5 L min^{-1}.

Desiccation experiments

Two types of desiccation experiments were conducted: (1) long-term, days, with thalli of *P. haitanensis* just collected and being desiccated indoors or outdoors; and (2) short-term, hours, with thalli pre-cultured indoors without desiccation for 1 week and then desiccated outdoors. All experimentation was conducted using immature thalli although some measurements were done using mature thalli as described below. The outdoor samples were maintained in a flow-through water bath for temperature control within a range of 20±2°C, while the indoor samples were maintained at the same temperature in an incubator.

Indoor long-term (days) experiment Thalli of *P. haitanensis* (0.6–0.8 g dry weight: DW) were placed in 500 mL spherical glass containers with the F-enriched seawater (500 mL), and

were cultured at 20°C and 220 μmol photons m^{-2} s^{-1} of PAR provided by fluorescent lamps (T5-28W, Tangye Lighting Technology, Shanghai, China) with a light-dark cycle of 12:12 h in the incubator. Six 500 ml glass containers were placed inside the incubator, triplicate containers for each treatment. In one treatment (non-desiccated), the thalli remained immersed in the culture medium during the whole experiment as control. In the other treatment (desiccated), all the thalli were taken out of the medium, placed on stainless steel nets and desiccated for 3 h every day (from 1100 to 1400 hours) during the light period in the incubator and then put back into the medium. The experiment lasted 4 days, and samples were taken at the beginning and at days 2 and 4 from each vessel to measure UV-absorbing compounds. For the desiccated treatment, samples were collected 3 h after re-submersion in medium (at 1700 hours), so that they were fully re-hydrated and the fresh weight determined.

Outdoor long-term (days) experiment This had the same basic design as the indoor experiment. The difference was that, during the desiccation period, thalli of *P. haitanensis* were exposed to natural solar radiation outside for 3 h around local noon (1100 to 1400 hours). Two radiation treatments were implemented to assess the differential effects of PAR and UVR: (1) PAB treatment, an Ultraphan 295 filter (UVT 100, Digefra, Munich, Germany) was placed above the samples so they received full spectrum of solar radiation during the desiccation period; and (2) P treatment, an Ultraphan 395 filter (UV Opak, Digefra, Germany) was placed above the samples to screen off solar UVR. Both treatments permitted desiccation as the air circulated freely below the filters. The transmission spectra of these filter foils are described elsewhere (Villafañe et al. 2003). Before and after the desiccation period, the thalli were maintained in the indoor incubator under the conditions mentioned above. Samples cultured as control were maintained always immersed in the medium inside the incubator. Some thalli without desiccation were cultured always under outdoor solar radiation (P, PAB) in 800-ml quartz tubes (inner diameter 5.4 cm, length 35 cm). Triplicate containers were implemented for each treatment and they were sampled every other day at 1700 hours during 10 days.

After 3 h desiccation indoor and outside, the water content of thalli had decreased to 50% and 10%, respectively. However, 15 min was enough for complete re-hydration of the desiccated samples.

Outdoor short-term (hours) experiment Thalli of *P. haitanensis* were pre-cultured under indoor conditions in an incubator without desiccation for 7 days to acclimate them to UVR-free conditions. After that period, the samples were taken outdoor and desiccated for 4 h under solar radiation around noon as described above (P and PAB treatments), while a control was continuously immersed under P treatment. Triplicate containers were implemented for each radiation treatment and samples were taken from each vessel for measurements of UV-absorbing compounds.

UVR impact on photosynthesis

In order to test the impact of UVR on *P. haitanensis*, experimentation was set up to measure UV-absorbing compounds and photochemical efficiency in relation to desiccation. The samples were exposed using two radiation treatments (P and PAB) for 1.5 h under a solar simulator (SOL 1200 W, Dr. Hönle AG, Munich, Germany) while submerged with aeration at a flow rate of about 0.4–0.5 L min^{-1} before and after 3 days pre-culture indoors with or without periodic (3 h) desiccation (20°C, 40 μmol photons m^{-2} s^{-1} of PAR with a light-dark cycle of 12:12 h). The samples received irradiances of 2.14, 65.7, and 282 (W m^{-2}) for UV-B, UV-A, and PAR, respectively. Recovery was followed during the following 4 h after the samples were transferred to dim white light (5–10 μmol photons m^{-2} s^{-1}). During the experiments the temperature was controlled by air conditioner at 20°C. The fluorometer (PAM-Water-EDF, Walz, Germany) was used to measure the effective quantum yield of photosystem II (PS II)($\Delta F/F'_m$, Genty et al. 1989), using the following equation:

$$\Delta F \Big/ F'_m = \left(F'_m - Ft\right) \Big/ F'_m,$$

where F_t is the current steady-state fluorescence which was measured with a modulated red light of 0.3 μmol photons m^{-2} s^{-1} and an actinic red light of 300 μmol photons m^{-2} s^{-1}, and F'_m is the maximal fluorescence yield of light-adapted samples which was measured after 0.8 s saturating pulse of approximately 6,000 μmol photons m^{-2} s^{-1}. The relative photosynthetic inhibition (%) due to UVR was calculated as:

$$Inh_{UVR}(\%) = (P_P - P_{PAB})/(P_P) * 100,$$

where P_P and P_{PAB} represented the effective quantum yield of samples exposed to PAR only and PAR + UV-A + UV-B. Recovery extent (%) of effective quantum yield in samples transferred into dim light was expressed as the percentage of the initial values before UVR stress.

Determination of UV-absorbing compounds and chlorophyll *a*

UV-absorbing compounds and chlorophyll *a* (chl *a*) were extracted by removing water drops from the thalli, weighing (fresh weight) and immersing them in 20 mL of

absolute methanol overnight at 4°C in darkness. After centrifugation at 5000 *g* for 10 min, the spectral absorption (250–750 nm) of the supernatant was scanned in a spectrophotometer. The concentration of UV-absorbing compounds was estimated on the basis of the absorption peak at 336 nm according to Dunlap et al. (1995). While triplicate samples were extracted, another set of triplicate samples was dried (85°C, overnight) to obtain dry weight and then the ratio of fresh weight to dry weight was determined. In order to determine the optimal amount of thalli to extract, a series of fresh weights ranging from 0.05 to 0.5 g were extracted in 20 mL of absolute methanol and the concentration of UV-absorbing compounds measured. The extraction efficiency decreased significantly when more than 0.3 g fresh weight of thalli was extracted; consequently, 0.1 g fresh mass of thalli was used for each extraction. The reader should be aware that the methodology used by us to estimate the presence of UV-absorbing compounds is not as sensitive as HPLC analyses. However, this does not invalidate our analyses and conclusionsl as the relative proportions determined in this study should also be maintained when using HPLC analyses.

In addition, when mature thalli were available, we determined the content of UV-absorbing compounds and chl *a* in different parts of the mature female and male thalli as explained above. The concentration of chl *a* was calculated according to Porra (2002).

Radiation measurements

PAR (400–700 nm) in the illuminated incubator was measured with an illuminometer (ST-80C, Photoelectric Instrument Factory of Beijing Normal University, Beijing, China). Outdoor incident solar radiation was monitored continuously with an ELDONET radiometer (Real Time Computers, Erlangen, Germany) permanently installed on the roof at Shantou University (116.59°E, 23.42°N). This instrument measures direct and indirect radiation (Ulbricht integrating sphere) at 1 s intervals in three different wavebands: PAR, UV-A (315–400 nm) and UV-B (280–315 nm) and records the averaged data at 1 min intervals.

Statistics

In most experiments, triplicate cultures or treatments were employed, but six PAM measurements were made for each determination of photochemical efficiency, and the data were expressed as mean±standard deviation (n=3~6). Statistical significance among different treatments was tested with *t*-test or ANOVA at a significant level of $p<0.05$. Tukey's post-hoc test was applied for comparison of means for the ANOVA.

Results

In the indoor experiments (Fig. 1), there was a continuous decrease in the concentration of UV-absorbing compounds as the experiment progressed. However, the desiccated *Porphyra haitanensis* thalli had a 119% higher content of UV-absorbing compounds than the submerged (non-desiccated) samples at day 2, but there were no significant differences ($p>0.05$) between treatments at day 4 (Fig. 1). Desiccation for 3 h indoors resulted in approximately 50% of water loss.

When *P. haitanensis* thalli were desiccated under solar radiation (Fig. 2), they had a higher concentration of UV-absorbing compounds than non-desiccated samples, and the significant differences between desiccated and non-desiccated samples were observed until day 8. The mean irradiances received by these thalli during 3 h desiccation were 1.09, 39.6, and 231 (W m^{-2}) for UV-B, UV-A, and PAR, respectively. Throughout this outdoor experiment, the control samples (always submerged indoors) and the non-desiccated samples that were exposed outside did not differ significantly ($p>0.05$) and they had a sharp and significant ($p<0.05$) decrease of UV-absorbing compound after 2 days from 7.87 to 2.27–3.06 mg g^{-1} DW. Desiccation under solar radiation for 3 h resulted in a water loss of about 90%, and the concentration of UV-absorbing compounds in desiccated samples was maintained during the first 4–6 days. The content of UV-absorbing compounds was 90–200% higher in the thalli desiccated under solar radiation than in those always submerged either indoors or outdoors. There were no significant differences between P and PAB treatments, regardless of desiccation or non-desiccation treatment (Fig. 2a, b).

In order to look at the potential short-term changes of UV-absorbing compounds, we exposed samples (desiccated and non-desiccated) for 4 h to solar radiation (Fig. 3). Due to the indoor pre-culture without desiccation for 7 days, the concentration of UV-absorbing compounds per unit dry

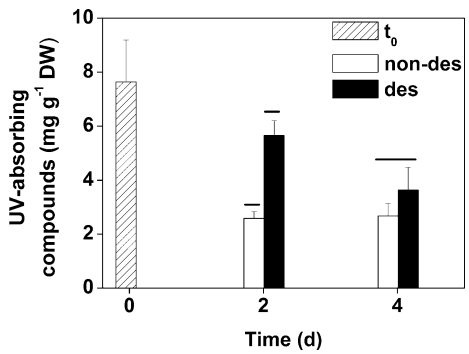

Fig. 1 Content of UV-absorbing compounds relative to dry weight (mg g^{-1} DW) in *Porphyra haitanensis* thalli during the indoor cultivation. Data are means±SD (n=3). *Horizontal lines* over the histograms indicate significant differences between treatments

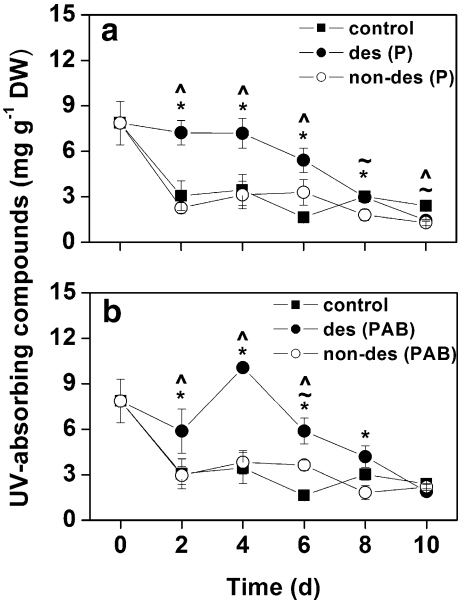

Fig. 2 Content of UV-absorbing compounds in *Porphyra haitanensis* thalli per unit dry weight during the long-term outdoor experiments. **a** Control and non-desiccated (*non-des*) and desiccated (*des*) samples exposed to PAR only (*P*); **b** control and non-desiccated (*non-des*) and desiccated (*des*) samples exposed to full spectrum of solar radiation (*PAB*). Data are means±SD (n=3). The experimental period was from 16 to 25 December 2004. ^ Indicates significant difference (p<0.05) between control and desiccated treatments, ~ between control and non-desiccated treatments, and * between desiccated and non-desiccated treatments

weight in *P. haitanensis* thalli had decreased to 2.36± 0.43 mg g^{-1} DW when this desiccation experiment began. After 4 h, the concentration showed no significant changes compared with the initial value, but in samples that were desiccated it was 50% (P) or 52% (PAB) higher than that in the non-desiccated thalli (P) (Fig. 3a, b). The mean irradiances received by the thalli were 0.76, 29.8, and 171 (W m^{-2}) for UV-B, UV-A, and PAR, respectively, during the 4 h exposure (Fig. 3c).

The UVR impact on photochemical efficiency of *P. haitanensis* decreased with increasing content of UV-absorbing compounds (Fig. 4). Fresh collected plants from the sea had a concentration of UV-absorbing compounds 7.46± 0.59 mg g^{-1} DW, and after 3 days of pre-culture indoors the value decreased significantly to 6.40±0.28 mg g^{-1} DW in the non-desiccated samples, whereas the value in the desiccated samples did not change significantly (8.14±0.24 mg g^{-1} DW). Photosynthetic inhibition due to UVR after 1.5 h exposure decreased with increasing content of UV-absorbing compounds (Fig. 4a). Recovery in the samples exposed to PAR only reached 82–83% of the values before exposure regardless of the amount of UV-absorbing compounds. Photochemical recovery in the samples under the PAB (PAR + UVR) treatment did not differ significantly (p>0.1) as compared to that under the P treatment at 7.46 and 8.14 mg g^{-1} DW concentrations of UV-absorbing compounds. However, the recovery was significantly lower in the PAB than in the P treatments exposed samples at 6.40 mg g^{-1} DW concentration of UV-absorbing compounds (Fig. 4b).

All the experiments mentioned above were conducted with immature thalli. However, as the season progressed we were able to collect mature thalli and these were analyzed to establish differences in the concentration of UV-absorbing compounds and chl *a* in different parts (Fig. 5). The contents of UV-absorbing compounds and of chl *a* per unit dry weight were 60–80% and 20% higher, respectively, in the middle and marginal parts than in the base of both female and male individuals (Fig. 5a, b). Significant differences between female and male individuals were found only in the marginal parts, the males containing 13% more UV-absorbing compounds but 20% less chl *a*.

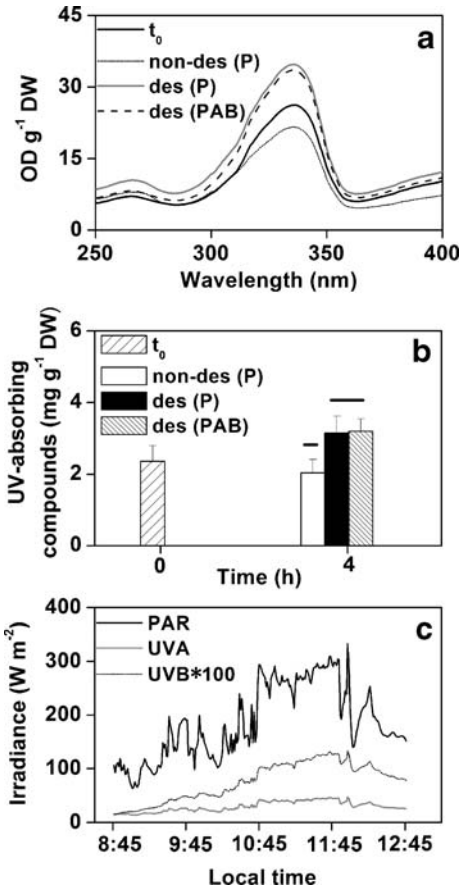

Fig. 3 Absorption characteristics of extractions from *Porphyra haitanensis* thalli and solar irradiance during the outdoor short-term experiments. (**a**) Absorption spectra of initial samples (t_0), non-desiccated (*non-des*) samples exposed to PAR only (*P*), desiccated (*des*) samples exposed to PAR only (*P*) and to full spectrum of solar radiation (*PAB*) for 4 h (OD g^{-1} DW). (**b**) Content of UV-absorbing compounds, expressed as mg g^{-1} DW. (**c**) Irradiances of solar PAR, UV-A and UV-B*100 (W m^{-2}) during 4 h exposure from 0845 to 1245 hours on 7 January 2005. Data are means±SD (n=3). *Horizontal lines* over the histograms indicate the differences among treatments. *Des*=Desiccated samples; *non-des*=non-desiccated samples

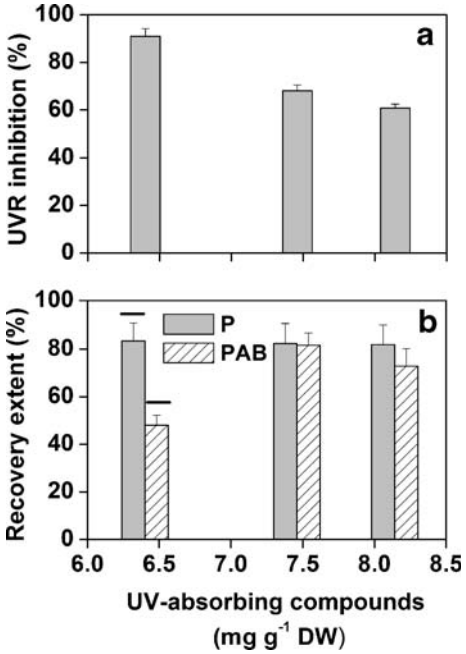

Fig. 4 Sensitivity to UVR of *Porphyra haitanensis* thalli with different content of UV-absorbing compounds, samples were taken at the beginning and after 3 days pre-culture indoor (non-desiccated and desiccated). (**a**) Relative photosynthetic inhibition (%) caused by UVR after 1.5 h exposure to solar simulated irradiance under PAR only (*P*) and full spectrum (*PAB*) as a function of content of total UV-absorbing compounds. (**b**) Recovery extent (%) after transferred into dim light for 4 h, expressed as percentage of their respective $\Delta F/F_m'$ before exposure. Data are means±SD (n=6). *Horizontal* lines over the histograms indicate the differences between treatments

Discussion

UV-absorbing compounds, mainly mycosporine-like amino acids (MAAs), have often been found to play a protective role against solar UVR (Conde et al. 2000; Karsten et al. 2005). However, it was also shown that these compounds did not increase in response to UVR and could not fully protect *Porphyra umbilicalis* and *Gracilaria cornea* against UVR (Gröniger et al. 1999; Sinha et al. 2000). We are aware that using spectrophotometric measurements of algal extracts could not identify MAAs, the main members of UV-absorbing compounds. However, the absorption characteristic of our *Porphyra haitanensis* thalli and the range of peak absorption pointed in the direction of MAAs. In addition, our spectra showed high consistency with those determined for other *Porphyra* species (Helbling et al. 2004), and the latter identified the UV-absorbing compounds as MAAs using HPLC techniques (Helbling et al. 2004; Korbee Peinado et al. 2004). Also, *Porphyra* spp. was reported to accumulate the highest concentrations of MAAs among the species of red algae studied so far (Gröniger et al. 1999; Hoyer et al. 2001). The method used in this study to quantify UV-absorbing compounds was based on the absorption peak at 336 nm (Dunlap et al. 1995), as this was a good estimator of total MAAs concentration, and therefore, the values calculated in this way should closely represent the contents of MAAs in *P. haitanensis*.

Induction of UV-absorbing compounds synthesis due to osmotic stress by adding sucrose to the medium was demonstrated in the cyanobacterium *Chlorogloeopsis* PCC 6912, although these compounds played an insignificant role in osmoregulation (Portwich and Garcia-Pichel 1999), while high concentrations of these compounds were suggested to regulate osmosis in another study (Oren 1997). And the formation of UV-screening compound, scytonemin, was induced by periodic desiccation in desiccation-tolerant cyanobacteria (Fleming and Castenholz 2007). In the present study, desiccation of *Porphyra haitanensis* thalli also resulted in a higher content of UV-absorbing compounds than in submersed thalli (Figs. 1, 2 and 3). A steady decrease of these compounds was also observed in *P. columbina* when the samples were immersed and exposed to solar radiation (Helbling et al. 2004). Our data presented here suggest that osmotic stress during desiccation might play a role in the induction of UV-absorbing compounds or perhaps in preventing them from degradation in *Porphyra* plants. Changes in salinity could also induce UV-absorbing compound synthesis, as found in the cyanobacterium *Chlorogloeopsis* PCC 6912 (Portwich and Garcia-Pichel 1999) and in the red alga *Devaleraea ramentacea* (Karsten et al. 2003). These changes of stress during the desiccation treatment have

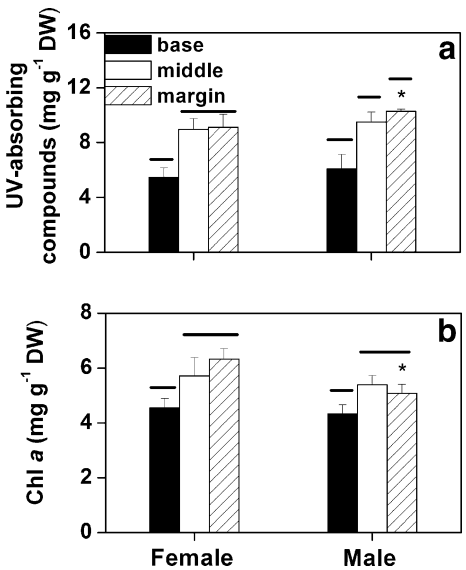

Fig. 5 Contents of UV-absorbing compounds (**a**) and chl *a* (**b**) expressed as mg g^{-1} DW in the basal, middle and marginal parts of male and female *Porphyra haitanensis* thalli. Data are means±SD (n=3). The *horizontal lines* over the histograms indicate the differences among parts, whereas the asterisks are the differences between female and male plants

been found to affect photosynthetic and respiratory rates of *Porphyra* plants (Gao and Aruga 1987). Reduced photosynthetic production during the desiccation could have been associated with some enhanced metabolic pathways related to production of UV-absorbing compounds. The enhanced accumulation of UV-absorbing compounds might also be attributed to stimulated nitrate and ammonium uptake after the desiccation during rehydration, as has been found in the red alga *Gigartina papillata*, the green alga *Enteromorpha intestinalis*, and in the brown algae *Fucus distichus* and *Pelvetiopsis limitata* (Thomas et al. 1987). Enriched ammonium concentrations have been shown to raise the contents of UV-absorbing compounds in *Porphyra* species (Korbee Peinado et al. 2004; Korbee et al. 2005). Desiccation treatment in the present study played a similar role as ammonium enrichment reported by Korbee et al. (2005) in slowing the degradation of UV-absorbing compounds in *Porphyra* species during the cultures.

Synthesis/degradation of UV-absorbing compounds may show high temporal variability as found in dinoflagellates: the synthesis occurred within hours in *Alexandrium excavatum* (Carreto et al. 1990), but within weeks in *Symbiodinium microadriaticum* (Banaszak and Trench 1995). In this study, UV-absorbing compounds in precultured *P. haitanensis* thalli were not actually enhanced by 4 h of desiccation (Fig. 3), but showed the increasing tendency, suggesting the induction potential of desiccation in this intertidal alga, while actual accumulation of UV-absorbing compounds might be found after another cycle of desiccation and resubmergence. Species of *Bangia* and *Porphyra* were found to accumulate UV-absorbing compounds not only in the field but also under long-term low-light laboratory conditions, suggesting a genetic rather an environmental control of these compounds (Gröniger et al. 1999; Karsten and West 2000; Hoyer et al. 2002). A specific UV-absorbing compound with an absorption peak at 324 nm was also assumed to be constitutive in the green alga *Prasiola* (Karsten et al. 2005). Thus, it was not strange to notice that content of UV-absorbing compounds in *P. haitanensis* thalli decreased to 20–30% of the initial value and then no further decrease in both indoor and outdoor experiments (Figs. 1 and 2) was observed.

The accumulation of UV-absorbing compounds was found to be dependent on both dose and wavelength of incident solar radiation, with higher accumulation of these compounds associated with high daily doses in *Chondrus crispus* (Karsten et al. 1998; Franklin et al. 2001). However, during both long- and short-term experiments in our study we did not find a UVR-dependent accumulation of UV-absorbing compounds (Figs. 2 and 3). The decrease in UV-absorbing compounds observed during the indoor culture might be caused by the decrease in irradiance from the natural conditions to the laboratory ones, for the synthesis of these compounds in *Chondrus crispus* was claimed to be induced by PAR and not by UVR (Franklin et al. 1999). PAR was shown to induce an increase of UV-absorbing compounds in dinoflagellates when grown at 200 μmol photons m^{-2} s^{-1} as compared to 20 μmol photons m^{-2} s^{-1} (Carreto et al. 1990). Nevertheless, the specific induction and the differential stability of different UV-absorbing compounds in *P. columbina* had been proposed (Korbee Peinado et al. 2004; Helbling et al. 2004), with the accumulation of shinorine being stimulated by UVR, while palythinol and palythine were mainly stimulated by PAR.

Most studies of the relationship between UV-absorbing compounds concentration and sensitivity to UVR in macroalgae focused on photosynthesis and found that species or individuals with higher contents of these compounds were more resistant to UVR stress (Maegawa et al. 1993; Karsten et al. 1999; Bischof et al. 2000). Higher contents of these compounds were associated with lower UVR-induced inhibition of photochemical efficiency in *P. leucosticta* and *P. umbilicalis* (Korbee et al. 2005). In our study, such a relationship was also exhibited in *P. haitanensis* thalli, with higher UVR-induced photosynthetic inhibition (Fig. 4a) and lower recovery (Fig. 4b) in samples containing relatively less UV-absorbing compounds. The accumulation of UV-absorbing compounds was not induced by UVR, but higher concentrations of these compounds provided more protection against UVR damage. It could be hypothesized that in *P. haitanensis* thallus desiccation might have induced the synthesis of UV-absorbing compounds or prevented them from degradation, and then these compounds could function as sunscreen by passively absorbing the deleterious wavelength of sunlight spectrum and protect the alga from photodamage. Desiccated *Fucus serratus* thalli showed less reduction in maximal quantum yield and O_2 evolution than those left submerged in tidal pools (Huppertz et al. 1990), though it was not clear whether protective sunscreen compounds were present or induced by desiccation. The protecting function of UV-absorbing compounds from high PAR was not obvious in our data, as in all samples exposed to PAR only the effective quantum yield decreased after 1.5 h exposure (data not shown) to 20–22% of the values before the exposure and recovered to 82–83% after 4 h in dim light (Fig. 4b), regardless of the amount of UV-absorbing compounds.

In *P. haitanensis* thalli consisting of heterogeneous cell types, we found differences of UV-absorbing compounds and chl *a* in the different parts, as well as between mature female and male individuals (Fig. 5). Basal parts of *Porphyra* spp. consist of rhizoidal cells and function as holdfast rather than photosynthetic organ, therefore the content of chl *a* was less than other parts. Photosynthetic pigment contents were also the lowest in the basal region

and increased toward the marginal region of *Ulva pertusa*, and photosynthetic capacity and photosynthetic efficiency normalized to fresh weight showed a progressive increase from the basal to marginal parts (Han et al. 2003). The tissue gradient in UV-absorbing compounds found in *Devaleraea ramentacea* was suggested to reflect different screening capacity between apices and basal tissue (Karsten et al. 1999). Apical tips of *Eucheuma*, which were exposed to high PAR and UVR, had higher concentrations of UV-absorbing compounds than the self-shaded basal regions (Wood 1989). Similarly, the basal parts of *P. haitanensis* thalli were shaded while farmed on the ropes during submersion and even covered during emersion by the upper leafy parts, especially in a cluster, thus less UV-absorbing compounds could be accumulated due to lower degree of desiccation or lower light stress. The carpogonia in marginal parts of the females might obtain more protection due to higher contents of UV-absorbing compounds in these area. Since higher tolerance to UVR in *P. haitanensis* thalli with more UV-absorbing compounds was demonstrated in this study, more UV-absorbing compounds in marginal parts of the males than corresponding parts of the females might provide more cellular protection for the spermatia against UVR, in order to guarantee enough healthy spermatia for fertilization.

Porphyra haitanensis is distributed in the upper parts of intertidal zone, experiencing periodic desiccation associated with tidal cycles. Dehydration due to desiccation at low tide regularly happens during daytime when the thalli are exposed to high levels of solar radiation. The ability of *Porphyra haitanensis* thallus to synthesize and maintain high cellular content of UV-absorbing compounds during such desiccation periods might allow it to cope with UVR and osmotic stress during daytime, thus providing a competitive advantage to inhabit the eulittoral zone.

Acknowledgements This work was supported by National Natural Science Foundation of China (Project No. 90411018), Ministry of Science and Technology ("863") and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). The experiments performed comply with the current laws of China.

References

Aguilera J, Jiménez C, Figueroa FL, Lebert M, Häder DP (1999) Effect of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis*. J Photochem Photobiol, B Biol 48:75–82

Banaszak AT, Trench RK (1995) Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. J Exp Mar Biol Ecol 194:233–250

Bischof K, Kräbs G, Hanelt D, Wiencke C (2000) Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? Helgoland Mar Res 54:47–52

Buma AGJ, van Hannen EJ, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. J Phycol 31:314–321

Carreto JI, Carignan MO, Daleo G, de Marco S (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J Plankton Res 12:909–921

Conde FR, Churio MS, Previtali CM (2000) The photoprotector mechanisms of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. J Photochem Photobiol, B Biol 56:139–144

Dring MJ, Wagner A, Lüning K (2001) Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. Plant Cell Environ 24:1153–1164

Dunlap WC, Yamamoto Y (1995) Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol 112:105–114

Dunlap WC, Rae GA, Helbling EW, Villafañe VE, Holm-Hansen O (1995) Ultraviolet-absorbing compounds in natural assemblages of Antarctic phytoplankton. Antarct J US 30:323–326

Fleming ED, Castenholz RW (2007) Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. Environ Microbiol 9:1448–1455

Franklin LA, Forster RM (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. Eur J Phycol 32:207–232

Franklin LA, Yakovleva I, Karsten U, Lüning K (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. J Phycol 35:682–693

Franklin LA, Kräbs G, Kuhlenkamp R (2001) Blue light and UVA radiation control the synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). J Phycol 37:257–270

Gao K, Aruga Y (1987) Preliminary studies on the photosynthesis and respiration of *Porphyra yezoensis* under emersed conditions. J Tokyo Univ Fish 47:51–65

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Gröniger A, Hallier C, Häder DP (1999) Influence of UV radiation and visible light on *Porphyra umbilicalis*: Photoinhibition and MAA concentration. J Appl Phycol 11:437–445

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Han YS, Han T (2005) UV-B induction of UV-B protection in *Ulva pertusa* (Chlorophyta). J Phycol 41:523–530

Han T, Han YS, Kain JM, Häder DP (2003) Thallus differentiation of photosynthesis, growth, reproduction, and UV-B sensitivity in the green alga *Ulva pertusa* (Chlorophyceae). J Phycol 39:712–721

Han T, Kong JA, Han YS, Kang SH, Häder DP (2004) UV-A/blue light-induced reactivation of spore germination in UV-B irradiated *Ulva pertusa* (Chlorophyta). J Phycol 40:315–322

Helbling EW, Gao K, Gonçalves RJ, Wu H, Villafañe VE (2003) Utilization of solar UV radiation by coastal phytoplankton assemblages off SE China when exposed to fast mixing. Mar Ecol Prog Ser 259:59–66

Helbling EW, Barbieri ES, Sinha RP, Villafañe VE, Häder DP (2004) Dynamics of potentially protective compounds in Rhodophyta

species from Patagonia (Argentina) exposed to solar radiation. J Photochem Photobiol, B Biol 75:63–71

Hoyer K, Karsten U, Sawall T, Wiencke C (2001) Photoprotective substances in antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. Mar Ecol Prog Ser 211:117–129

Hoyer K, Karsten U, Wiencke C (2002) Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. Mar Biol 141:619–627

Huppertz K, Hanelt D, Nultsch W (1990) Photoinhibition of photosynthesis in the marine brown alga *Fucus serratus* as studied in field experiments. Mar Ecol Prog Ser 66:175–182

Karsten U, West JA (2000) Living in the intertidal zone - seasonal effects on heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales). J Exp Mar Biol Ecol 254:221–234

Karsten U, Franklin LA, Lüning K, Wiencke C (1998) Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). Planta 205:257–262

Karsten U, Bischof K, Hanelt D, Tüg H, Wiencke C (1999) The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). Physiol Plant 105:58–66

Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. Polar Biol 26:249–258

Karsten U, Friedl T, Schumann R, Hoyer K, Lembcke S (2005) Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). J Phycol 41:557–566

Klisch M, Sinha RP, Häder DP (2002) UV-absorbing compounds in algae. Curr Top Plant Biol 3:113–120

Korbee N, Huovinen P, Figueroa FL, Aguilera J, Karsten U (2005) Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two *Porphyra* species (Bangiales, Rhodophyta). Mar Biol 146:645–654

Korbee Peinado N, Abdala Díaz RT, Figueroa FL, Helbling EW (2004) Ammonium and UVR stimulate the accumulation of mycosporine-like amino acids (MAAs) in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. J Phycol 40:248–259

Lud D, Buma AGJ, van de Poll W, Moerdijk TCW, Huiskes AHL (2001a) DNA damage and photosynthetic performance in the Antarctic terrestrial alga *Prasiola crispa* ssp. *antarctica* (Chlorophyta) under manipulated UV-B radiation. J Phycol 37:459–467

Lud D, Huiskes AHL, Moerdijk TCW, Rozema J (2001b) The effects of altered levels of UV-B radiation on an Antarctic grass and lichen. Plant Ecol 154:87–99

Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jpn J Phycol 41:207–214

Nakamura H, Kobayashi J, Hirata Y (1982) Separation of mycosporine-like amino acids in marine organisms using reverse-phase high performance liquid chromatography. J Chromatogr 250: 113–118

Oliver MJ, Velten J, Wood AJ (2000) Bryophytes as experimental models for the study of environmental stress tolerance: *Tortula ruralis* and desiccation-tolerance in mosses. Plant Ecol 151:73–84

Oren A (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiol J 14:231–240

Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol Lett 269:1–10

Pakker H, Beekman CAC, Breeman AM (2000) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). Eur J Phycol 35:109–114

Porra RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. Photosynth Res 73:149–156

Portwich A, Garcia-Pichel F (1999) Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. Arch Microbiol 172:187–192

Sinha RP, Klisch M, Gröniger A, Häder DP (2000) Mycosporine-like amino acids in the marine red alga *Gracilaria cornea* - effects of UV and heat. Environ Exp Bot 43:33–43

Suh HJ, Lee HW, Jung J (2003) Mycosporine glycine protects biological systems against photodynamic damage by quenching singlet oxygen with a high efficiency. Photochem Photobiol 78:109–113

Thomas TE, Turpin DH, Harrison PJ (1987) Desiccation enhanced nitrogen uptake rates in intertidal seaweeds. Mar Biol 94:293–298

van de Poll WH, Eggert A, Buma AGJ, Breeman AM (2002) Temperature dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. Eur J Phycol 37:59–68

Villafañe VE, Sunbäck K, Figueroa FL, Helbling EW (2003) Photosynthesis in the aquatic environment as affected by ultraviolet radiation. In: Helbling EW, Zagarese HE (eds) UV Effects in aquatic organisms and ecosystems. The Royal Society of Chemistry, Cambridge, pp 357–397

Wood WF (1989) Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. Aquat Bot 33:41–51

Zou D, Gao K (2002a) Effects of desiccation and CO_2 concentrations on emersed photosynthesis in *Porphyra haitanensis* (Bangiales, Rhodophyta), a species farmed in China. Eur J Phycol 37:587–592

Zou D, Gao K (2002b) Photosynthetic bicarbonate utilization in *Porphyra haitanensis* (Bangiales, Rhodophyta). Chinese Sci Bull 47:1629–1633

Zou D, Gao K (2004) Exogenous carbon acquisition of photosynthesis in *Porphyra haitanensis* (Bangiales, Rhodophyta) under emersed state. Prog Nat Sci 14:138–144