

BLUE LIGHT AND UV-A RADIATION CONTROL THE SYNTHESIS OF MYCOSPORINE-LIKE AMINO ACIDS IN *CHONDRUS CRISPUS* (FLORIDEOPHYCEAE)¹

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The induction of UV-absorbing compounds known as mycosporine-like amino acids (MAAs) by red, green, blue, and white light (43% ambient radiation greater than 390 nm) was examined in sublittoral *Chondrus crispus* Stackh. Fresh collections or long-term cultures of sublittoral thalli, collected from Helgoland, North Sea, Germany, and containing no measurable amounts of MAAs, were exposed to filtered natural radiation for up to 40 days. The MAA palythine (λ_{\max} 320 nm) was synthesized in thalli in blue light to the same extent observed in control samples in white light. In contrast, thalli in green or red light contained only trace amounts of MAAs. After the growth and synthesis period, the photosynthetic performance of thalli in each treatment, measured as pulse amplitude modulated chlorophyll fluorescence, was assessed after a defined UV dose in the laboratory. Thalli with MAAs were more resistant to UV than those without, and exposure to UV-A+B was more damaging than UV-A in that optimal (F_v/F_m) and effective (ϕ_{II}) quantum yields were lower and a greater proportion of the primary electron acceptor of PSII, Q, became reduced at saturating irradiance. However, blue light-grown thalli were generally more sensitive than white light control samples to UV-A despite having similar amounts of MAAs. The most sensitive thalli were those grown in red light, which had significantly greater reductions in F_v/F_m and ϕ_{II} and greater Q reduction. Growth under UV radiation alone had been shown previously to lead to the synthesis of the MAA shinorine (λ_{\max} 334 nm) rather than palythine. In further experiments, we found that preexposure to blue light followed by growth in natural UV-A led to a 7-fold increase in the synthesis of shinorine, compared with growth in UV-A or UV-A+B without blue light pretreatment. We hypothesize that there are two photoreceptors for MAA syn-

thesis in *C. crispus*, one for blue light and one for UV-A, which can act synergistically. This system would predispose *C. crispus* to efficiently synthesize UV protective compounds when radiation levels are rising, for example, on a seasonal basis. However, because the UV-B increase associated with artificial ozone reduction will not be accompanied by an increase in blue light, this triggering mechanism will have little additional adaptive value in the face of global change unless a global UV-B increase positively affects water column clarity.

Key index words: blue light; chlorophyll fluorescence; *Chondrus crispus*; MAA; macroalgae; mycosporine-like amino acids; photosynthesis; UV-B

Abbreviations: F_o , chlorophyll fluorescence of open PSII centers; F_m , F_v , maximum and variable chlorophyll fluorescence after dark incubation, respectively; F_v/F_m , optimal quantum yield; F_s , F_m' , steady state and maximum chlorophyll fluorescence in the light, respectively; F_o , minimum chlorophyll fluorescence in darkness immediately after a saturating flash; ϕ_{II} , effective quantum yield in the light; MAA, mycosporine-like amino acid; PUR, photosynthetically usable radiation; Q, primary electron acceptor of PSII; q_p , photochemical quenching of fluorescence; UVR, ultraviolet radiation

The mycosporine-like amino acids (MAAs), a class of at least 19 compounds with absorbance between 310 nm and 360 nm (Shibata 1969, Tsujino et al. 1978, 1980, Nakamura et al. 1982, Karentz et al. 1991), have long been proposed to protect organisms from damage by UV radiation, particularly UV-B. Occurring in a number of taxonomically diverse organisms, particularly corals (Dunlap et al. 1986, Dunlap and Shick 1998), microalgae (Jeffrey et al. 1999), and rhodophyte algae (Sivalingam et al. 1974, Karentz et al. 1991, Karsten et al. 1998b, Hoyer et al. 2001), the concentration of MAAs in tissues is often correlated with irradiance, with variation observed at scales from the level of irradiance microclimate in algal turfs to the

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water column depth (e.g. Sivalingam et al. 1974, Beach and Smith 1996, Karsten et al. 1999, Karsten and Wiencke 1999, Hoyer et al. 2001). Interest in the biochemistry and physiology of MAAs and MAA-containing organisms has grown recently as reductions in the stratospheric ozone concentration and a correlated increase in the amount of UV-B at the Earth's surface have been reported.

MAAs are chemical derivatives of a mycosporine cyclohexenone or cyclohexenimine chromophore conjugated with one or two of several different amino acids. The MAA biosynthetic pathway is not conclusively known, but a recent study of the synthesis of MAAs in coral indicates that synthesis proceeds via early steps in the shikimic acid pathway (Shick et al. 1999), the process by which precursors of mycosporine are synthesized in fungi (Favre-Bonvin et al. 1987), and the general pathway for synthesis of aromatic amino acids. The basis of the diversity of amino acid substitutions is not clear, but selective hydrolysis of two MAAs, shinorine and porphyra-334, to yield a third, mycosporine-glycine, by the marine bacterium *Vibrio Harveyi* (Johnson and Shunk) Baumann et al. has been demonstrated (Dunlap and Shick 1998).

Macroalgae containing MAAs normally grow in shallow or eulittoral environments where changes in water clarity are stochastic. Additionally, these environments can impose multiple stresses on organisms in addition to UV radiation (UVR), for example high PAR, desiccation, and nutrient limitation. Comparison of the integrity of cellular components and physiological processes in algae that contain MAAs with those without shows that the former are generally more resistant to UV-B-induced damage, though the degree of protection varies (Garcia-Pichel et al. 1993, Lesser 1996, Neale et al. 1998, Franklin et al. 1999). Besides having a role in UV screening, several MAAs also demonstrate antioxidant properties (Dunlap and Shick 1998), and MAAs in blue green algae may act as compatible solutes (Oren 1997, but see Portwich and Garcia-Pichel 1999), suggesting that MAAs may be important compounds for resistance to more than just UV stress.

Perhaps it is not surprising that MAA absorption in the UV range does not necessarily mean that the presence of UV-B in the environment is a prerequisite for their synthesis. Although UV-A and particularly UV-B are required for MAA synthesis in some organisms, other species contain high levels of MAAs without ever having been exposed to UVR (c.f. Carreto et al. 1990, Hannach and Sigleo 1998, Karsten et al. 1998a, Franklin et al. 1999, Jeffrey et al. 1999, Karsten et al. 1999, Karsten and Wiencke 1999, Portwich and Garcia-Pichel 1999, Shick et al. 1999). In other cases, MAAs are not synthesized when the alga is exposed to either higher PAR or UV (Hoyer et al. 2001). These diverse responses raise questions about the underlying biochemical trigger for induction, the nature of the receptor, and the taxonomic commonality of the induction process.

Using natural radiation and filters differentially transparent to UV and PAR, we recently showed that

identical patterns of MAA synthesis in the rhodophyte *C. crispus* can be induced by PAR alone or PAR containing either UV-A or UV-A plus UV-B (Franklin et al. 1999). Exposure to surface levels of UVR without PAR can also induce MAA synthesis in *C. crispus* (Karsten et al. 1998a), but these two conditions differ markedly in the relative proportion of the various MAAs produced. Only when PAR is present are MAAs produced that are quantitatively and qualitatively similar to those normally seen in eulittoral *C. crispus* populations. Induction by PAR follows a distinct pattern, with initial synthesis of the MAA shinorine (λ_{\max} 334 nm), followed by synthesis of predominantly palythine (λ_{\max} 320 nm) with a concomitant decline in shinorine content, leading to the proposal that interconversion is a key step in the synthesis of the main *C. crispus* MAA, palythine. In contrast, exposure to UVR alone led primarily to the synthesis of shinorine and lower amounts of palythine, perhaps representing the presence of two distinct photoreceptors. Results were more difficult to interpret because the PAR cut-off filter used in both experiments transmitted significant long-wave UV-A (50% transmittance at 380 nm).

We continued to investigate induction of MAA synthesis in *C. crispus* by PAR, by exposing sublittoral and laboratory-cultured thalli to white, red, green, and blue light filtered from natural irradiance, using filters with less than 5% transmittance at 380 nm. We also investigated the possible relationship between induction by PAR and UV, using sequential treatments. We show that synthesis of MAAs, particularly palythine, is preferentially induced by blue light, and blue light and UV-A interact to boost synthesis of shinorine. In a controlled laboratory test, photosynthesis in thalli with the highest levels of MAAs was more protected from UV-A than in thalli without MAAs, but there were unexpected differences in the photosynthetic response of MAA-containing samples to UV-B. This difference depended on the wavelength of light used to induce MAA synthesis. Of all conditions tested, prior exposure to white light led to the most effective protection and recovery from UV stress.

MATERIALS AND METHODS

Plant material and treatment conditions. *Chondrus crispus* was collected from the sublittoral zone (6 m below mean low water of spring tides) at Helgoland, Germany, a rocky island in the southeastern North Sea. Unialgal cultures were established from fertile gametophytes and grown in Provosoli's Enriched Seawater medium (Starr and Zeikus 1993) for four generations at 50 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Daylight Deluxe, Osram, Germany), 16:8 day:night cycle, 18° C. One experiment was performed with 2–3 cm tetrasporophytes from these cultures. Other experiments were performed with fresh sublittoral (6 m) field material collected from the same location. Fresh material was cleaned of epiphytes and held overnight indoors in running seawater, before exposure to the treatment conditions.

The spectral response of MAA induction was tested using natural irradiance filtered to obtain four light conditions (Fig. 1): red (Lee, Andover, England), green (Kodak, Rochester, NY), blue (Lee), and white light (PAR reduced to 43% of surface ambient by neutral density screening, UV removed by Ultraphan 395 foil, Digefta GmbH, Munich, Germany). The fil-

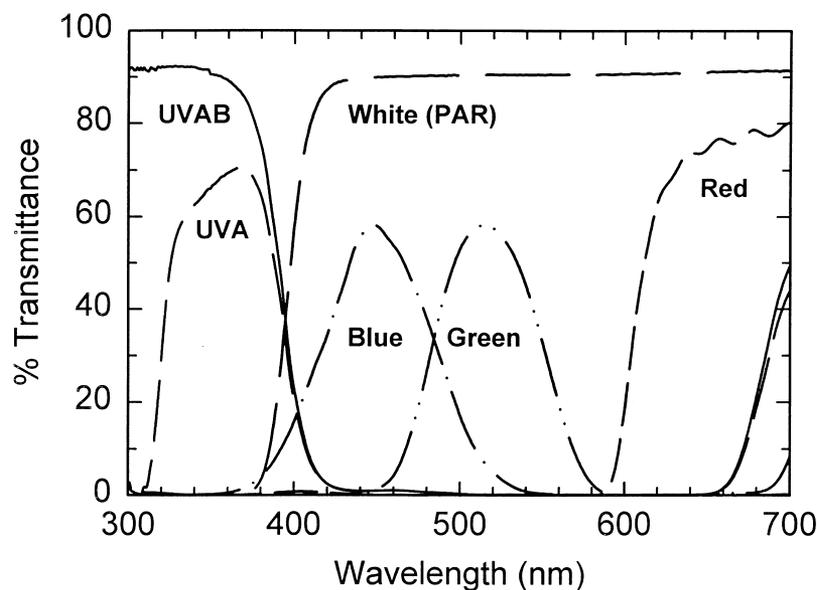


FIG. 1. Transmittance spectra of the colored foils and total UVR-blocking (white), PAR-blocking (UVAB), and UV-B-blocking (UVA) filters used during induction of MAAs.

ters and screens were selected to give as equal an energy distribution over the specific part of the spectrum as possible (Table 1). At midday on a clear day in June, the total natural energy transmitted in the 350- to 700-nm range was nearly equal among the blue, green, and red treatments. Both blue and white filters had small tails in the long UV-A range, 4% and 3% transmittance at 380 nm, respectively. The red and blue filters also transmitted equal amounts of far red (700–800 nm). Although the white and red filters transmitted equal amounts of red light (590–700 nm), the white treatment had 50% more blue light (350–500 nm) than the blue treatment, and the total energy transmitted in the white treatment (350–700 nm) was three times greater than any of the other filters. Natural light spectra were measured with an LI-1800UW spectroradiometer (Li-Cor, Lincoln, NE).

The filters and screens were wrapped around 65 × 20 cm diameter acrylic tubes (Plexiglas XT, Röhm GmbH, Darmstadt, Germany), open at each end for water flow. Thalli were attached in rows by fine silicone tubing to clear acrylic plates suspended lengthwise in the Plexiglas tubes. The tubes were suspended horizontally 2 cm below the water surface in a tank 1 × 1.5 × 0.5 m deep, containing 750 L seawater at 17.5°C (± 1.5°C). Fresh seawater was directed into the end of each tube at approximately 5 L·min⁻¹. Plants were put into and collected from the treatments in the evening. Results of the experiment with cultured thalli were confirmed by a replicate experiment using

fresh sublittoral field material. Results from the cultured material are presented.

In early autumn, the possible interaction of blue light and UV was tested by applying sequential light treatments. Sublittoral thalli were treated for 2 weeks in the blue tubes and then moved to small polyvinyl chloride holders covered with a UG5 glass filter (Schott Glaswerke, Mainz, Germany) that blocked all wavelengths greater than 400 nm (Fig. 1). In addition, a piece of 320-nm-long pass foil (Folex, Dr. Schleusner GmbH, Dreierich, Germany) was added to remove UV-B from the UVR spectrum (UV-A-treated samples), or a piece of 295-nm-long pass foil (Ultraplan 295, Lonza-Folien, Weil am Rhein, Germany) was added for a full UV-A+B treatment. Further details on these filter and polyvinyl chloride holder combinations are given in Karsten et al. (1998a). The change in experimental setup was necessary because no flexible PAR-blocking filters were available to wrap around the tubes. Therefore, comparisons were made to three sets of control thalli: 1) those held at the same period of time under a blue filter in these holders (no blue pretreatment), or 2) those held under a UG5 filter (no blue pretreatment), or 3) blue pretreated thalli that then remained in blue light but in the new holders.

Light conditions at Helgoland during these periods were monitored continuously by a PUV-500 radiometer (Biospherical Instruments, Inc., San Diego, CA) mounted permanently on the roof of the research laboratory.

Analysis of MAAs and photosynthetic pigments. Several times during the treatment period, samples were collected for HPLC and spectral analysis of MAAs. Samples were divided in half longitudinally, weighed, and then dried in silica gel. One half of each sample was extracted for 2 h in 25% aqueous methanol (vol/vol) at 45°C, and the extracts were scanned spectrophotometrically (Schimadzu UV-2101PC, Kyoto, Japan). The other half was extracted in an identical manner, and the MAAs were quantified by HPLC (Karsten and Garcia-Pichel 1996, Karsten et al. 1998a). Briefly, MAAs were separated on a Waters HPLC system (Waters, Milford, MA) fitted with a Knauer Spherisorb RP-8 column (Knauer, Berlin, Germany). The mobile phase was 25% aqueous methanol (vol/vol) plus 0.1% acetic acid (vol/vol), run isocratically. Peaks were detected at 335 nm, and absorption spectra from 290 nm to 400 nm were recorded for each peak detected. MAAs were identified by spectra, retention time, and, in some cases, by cochromatography with standards kindly provided by D. Karentz (University of California, San Francisco, CA). Quantification was made using published extinction coefficients (Tsujino et al. 1980, Dunlap et al. 1986, Karsten et al.

TABLE 1. Comparison of the energy transmittance of the colored filters and the white light (PAR reduced with two layers of neutral density screening) treatments to the incident surface irradiance.

Filter color	Irradiance, 12 ⁰⁰ clear day, W·m ⁻²			
	350–500 nm	425–600 nm	590–700 nm	350–700 nm
Blue	52.7			52.7
Green		55.1		55.1
Red			50.1	50.1
White	74	90	51	145
Incident surface	172	208	118	337

UV radiation was removed from the white light treatment with a UV-blocking filter. Measurements were made on a clear day at midday, the day before the start of the first experiment, using a LI-1800UW spectroradiometer.

1998a). Results of the HPLC analysis are expressed as $\text{mg}\cdot\text{g}^{-1}$ dry weight (DW). Additional samples were collected for extraction of chl *a* (Porra et al. 1989) and phycobiliproteins (Beer and Eshel 1985).

UV stress test. Before and at the end of the month-long treatment, samples were tested for UVR sensitivity by means of a standard 3-h UV "stress test" applied in the laboratory. Although the quantity of UV-B in the test was set to an ecologically relevant level, the test was not designed to mimic the solar spectrum in the field. Rather, this method has been previously shown to be effective in assessing the relative degree of acclimation to UVR (Franklin et al. 1999). In this test, we used 320 nm as the division between UV-B and UV-A because of the spectral characteristics of our filters. The test was applied in a temperature-controlled water bath (18°C) to 1.5-cm-long pieces of similar shape cut from the thallus tips. The UV stress treatments were made using UVA 340 fluorescent lamps (Q-Panel Co., Cleveland, OH), in combination with 20 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR from daylight fluorescent lamps (Daylight Deluxe). The emission spectrum of the UVA 340 lamps was similar to the solar spectrum at wavelengths less than 345 nm and contained no radiation below 295 nm. Lamp spectra were measured using the LI-1800UW spectroradiometer, and the distance between the lamps and the samples was adjusted to approximate the unweighted UV-B irradiance at midday on a typical summer day on Helgoland. Irradiances were as follows: UV-B (300–320 nm) 1.4 $\text{W}\cdot\text{m}^{-2}$, UV-A (320–400 nm) 20 $\text{W}\cdot\text{m}^{-2}$, and PAR (400–700 nm) 20 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The effective (weighted) UV-B irradiance was 0.139 $\text{W}\cdot\text{m}^{-2}$, calculated using Caldwell's generalized plant action spectrum (PAS_{300}) (Björn and Murphy 1985). Because MAAs have substantial absorption in the UV-A spectrum, the effects of UV-A and UV-B were tested separately. One tip of each thallus was exposed to both UV-A+B in the stress test, whereas another tip was exposed only to UV-A, the UV-B having been removed by a sheet of the 320-nm-long pass foil.

Sensitivity of the algae to the test was indicated by differences in chlorophyll fluorescence characteristics measured three times: before and after the test and after 3 h recovery at 20 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR. Fluorescence was measured with a portable Pulse Amplitude Modulation (PAM-2000) fluorometer (H. Walz, Effeltrich, Germany). The optimal quantum yield, F_v/F_m ($F_v = F_m - F_o$), was determined by the following procedure: After 10 min of darkness, a 10-s pulse of dim far red light was applied, the F_o value was recorded, and F_m was determined by a saturating flash (Schreiber et al. 1986, 1995). Proceeding immediately, 3 min of 35 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from the PAM 2000 internal light-emitting diode array (emission maximum 655 nm) was applied to activate photosynthetic dark reactions, and then a light response curve of fluorescence was

recorded from 7 to 350 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At each irradiance, the steady state fluorescence level (F_s) was allowed to stabilize and then a saturating flash was applied (F_m'), followed by 5 s of darkness (F_o'). The effective quantum yield, $\phi_{II} = (F_m' - F_s) / F_m'$ (Genty et al. 1989), and reduction status of the primary PSII acceptor, $Q, 1 - qP = 1 - (F_m' - F_s) / (F_m' - F_o')$ (Bilger and Björkman 1990), were calculated. Samples were held in a stirred temperature-controlled (18°C) chamber during the measurements. Irradiance was measured using a cosine-corrected quantum sensor (Li-Cor).

Statistical analyses. Under given conditions, differences in photosynthetic pigments and fluorescence characteristics among the red, green, blue, and white samples were assessed by analysis of variance (Genstat 5 Release 3.1, Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK).

RESULTS

Irradiance and general appearance of thalli. The cumulative irradiance for the treatment periods can be found in Table 2. Only those wavelengths relevant to the treatments are presented. Many days in these periods were cloudy or completely overcast. By late September, global irradiance had declined substantially from the summer values, and approximately half the amount of irradiance was received in a given period as during the summer.

The major photosynthetic light-harvesting pigments in *C. crispus* are the phycobiliproteins, which absorb light between 460 nm and 570 nm. Though not measured directly, thalli under the blue filter treatment would have initially received the least amount of photosynthetically usable radiation (PUR) (Morel 1978) on a quantum basis and those in the white light the most. After 40 days, the absolute concentrations of photosynthetic pigment in all treatments changed significantly relative to the initial condition, but these changes affected the ratio of phycobiliproteins to chl *a* only in samples under blue light (Table 3), apparently in response to low PUR. In these samples, the ratio doubled due to increases in both phycoerythrin and phycocyanin, and samples appeared almost purple. The phycoerythrin content of green and red light acclimated samples was similar to white light controls

TABLE 2. Cumulative irradiance values summed over the period when the spectral response of MAA induction was tested (19 July to 27 June 1998) and when the effect of a prior exposure to blue light was tested (3 September to 5 October 1998).

Experiment, dates and days of treatment	305 nm	320 nm	340 nm	380 nm	400–700 nm ($\text{mol}\cdot\text{m}^{-2}$)
	(kJ·m ⁻² ·nm ⁻¹)				
Spectral response of MAA induction					
19 June to 27 July 1998					
7 d W, B, G, R				99.3	247
14 d W, B, G, R				214.3	531
29 d W, B, G, R				403.5	980
40 d W, B, G, R				568.9	1404
Sequential blue and UV treatment					
3 September to 5 October 1998					
18 d B pretreatment				111.6	263
2 d UV or B	0.18	2.6	5.2	8.0	16
3 d UV or B	0.47	7.1	14.3	22.11	55
7 d UV or B	0.76	14.0	29.1	44.22	106
14 d UV or B	1.20	25.3	53.7	82.28	195

The number of days refers to the total length of time a sample set was under the treatment conditions. Measurements were made with a Biospherical PUV-500 radiometer mounted on the roof of the research building on Helgoland. Actual amounts of radiation received were reduced as indicated by the filter spectra in Figure 1. W, white; B, blue; G, green; R, red.

TABLE 3. Photosynthetic pigments in *Chondrus crispus* before and after 40 days of acclimation to white, blue, green, or red light.

Treatment	Phycoerythrin	Phycocyanin	Chl <i>a</i>	Phycobiliproteins/chl <i>a</i>
	$\mu\text{g}\cdot\text{mg}^{-1}$ fresh weight			
Initial	1.51 (0.20) ^{a,b}	0.21 (0.02) ^a	0.41 (0.06) ^{a,b}	4.26 (0.87) ^a
White	1.30 (0.23) ^{a,b}	0.19 (0.02) ^a	0.31 (0.04) ^{c,d}	4.86 (0.73) ^a
Blue	2.81 (0.36) ^c	0.33 (0.04) ^c	0.37 (0.06) ^{b,c}	8.75 (1.94) ^b
Green	1.68 (0.40) ^b	0.22 (0.03) ^a	0.44 (0.01) ^a	4.33 (0.95) ^a
Red	1.08 (0.31) ^a	0.28 (0.02) ^b	0.28 (0.03) ^d	4.87 (1.34) ^a

Averages (SD), $n = 4$. Statistically different values are marked by different superscripts ($P < 0.05$).

but higher under blue light. The concentration of phycocyanin increased from equal amounts in the white and green treatments to higher in the red and even higher in the blue treatments. The chl *a* content was highest in green light samples, progressively decreasing in blue, white, and red samples.

Growth rates were estimated from the change in total biomass from one sampling time to the next. Over the course of 40 days, the growth rate of samples in white light was approximately twice that of those in red or green light and seven times higher than in blue light (data not shown). This agrees with the hypothesis of low PUR in the blue treatment and suggests that phycobiliprotein synthesis under this condition did not compensate for the lower PUR in terms of growth. Microscopic observation revealed that red light-acclimated samples developed very thick cell walls as compared with all other treatments (not shown).

Spectral response of MAA induction. Before transfer to the treatment conditions, extracts from initial samples, either cultured or collected fresh from the sublittoral, contained no clear absorbance peaks in the UV-A or UV-B range (Fig. 2). Differential UV absorbance among the red, green, blue, and white treatments was observed within 7 days of the start of the experiment. Representative spectra of extracts obtained

from thalli of equal weight suggested that, relative to the initial condition, there was similar synthesis of UV-absorbing compounds in white and blue treatments, but a loss of compounds under red and green light. Thalli in the white or blue treatments contained substances absorbing strongly between 320 and 370 nm, with broad peaks at 330 nm and 360 nm. Differences between white and blue versus green and red were maintained over the remainder of the 40-day experimental period.

The presence of MAAs was confirmed by HPLC analysis of samples collected throughout the duration of the experiment (Fig. 3). After the first week, thalli in both white and blue treatments contained primarily palythine, though samples in white light contained approximately four times as much on a DW basis. Samples in white light also contained small amounts of shinorine. The palythine content in blue light-treated thalli rose nearly linearly over the course of 40 days to $0.8 \text{ mg}\cdot\text{g}^{-1}$ DW, and at the end of the experiment there was no significant difference between samples in the blue or white treatment. Induction was light saturated at the levels used in the blue treatment, because the 50% more blue light in the PAR treatment (Table 1) did not result in any greater MAA content. In contrast, there was less than $0.15 \text{ mg}\cdot\text{g}^{-1}$

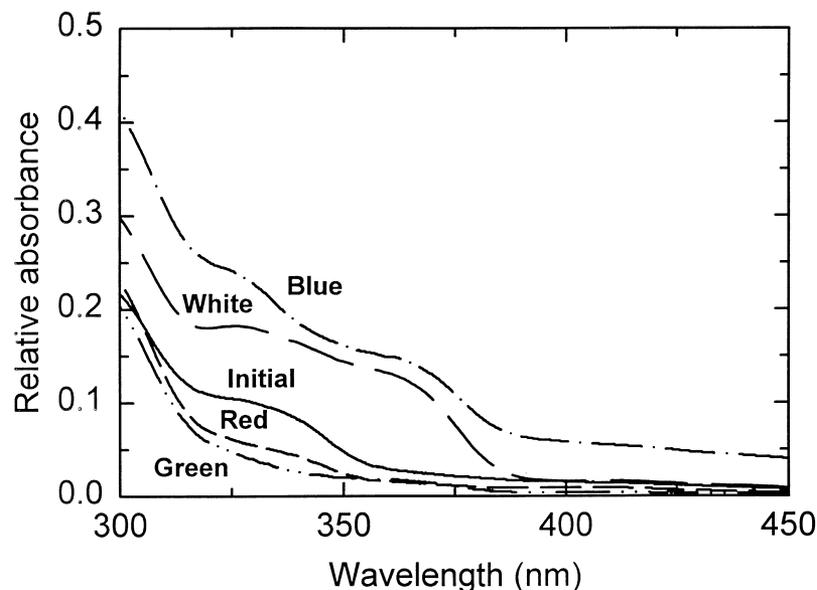


FIG. 2. Extracts of UV-absorbing compounds from *Chondrus crispus* after 1 week in the blue, green, red, or white filter treatments. Samples were of equal fresh weight, extracted in equal volumes of 25% aqueous methanol.

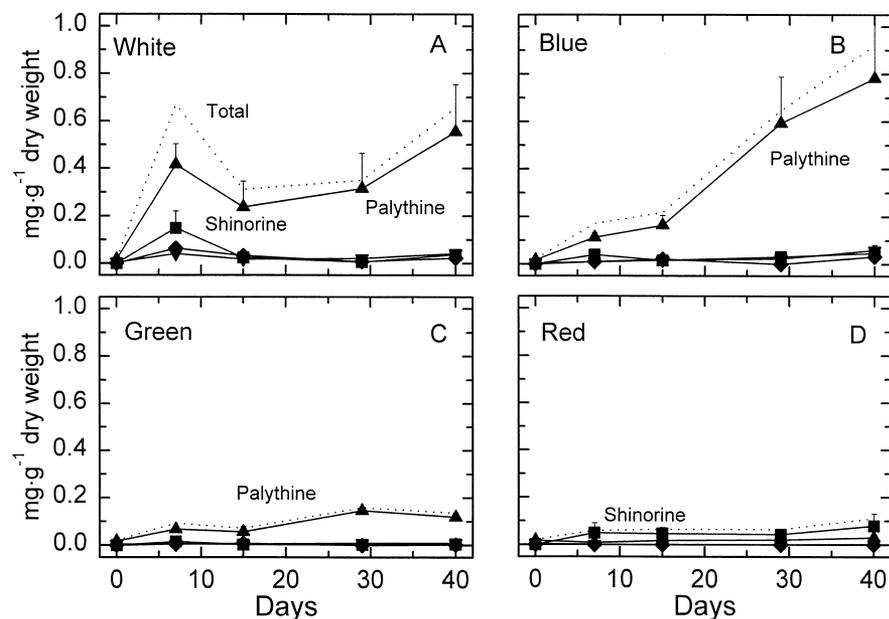


FIG. 3. Time course of the accumulation of MAAs in *Chondrus crispus* under (A) PAR, (B) blue, (C) green, or (D) red filter treatments, expressed on a thallus dry weight basis. The major MAAs are identified on the panels; only trace amounts of asterina-330 and palythene were detected. The dotted line is the sum of all MAAs. Means \pm SD, $n = 5$.

DW palythine measured in the green treatment and less than $0.1 \text{ mg}\cdot\text{g}^{-1}$ DW shinorine in the red treatment at any time within the 40 days. Only trace amounts of asterina-330 and palythene were detected in any treatment at any time.

Response to a standard UV stress before synthesis of MAAs. PAM chlorophyll fluorescence characteristics of cultured *C. crispus* are shown in Figure 4, before and after the 3-h UV-A or the UV-A+B stress test and after 3 h recovery in low PAR. Before exposure to any UV stress, F_v/F_m was 0.65 (Fig. 4A, value at 0 irradiance). Upon exposure to increasing irradiance in a light response curve, ϕ_{II} declined from 0.65 to 0.17 as more excitation energy was received by the thallus than was required for electron transport. At the same time, the reduction status of Q increased in response to increasing irradiance, from a fully oxidized state at low irradiance to just under 60% reduction at $380 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 4D). After exposure to 3 h UV-A or UV-A+B in low background PAR, both F_v/F_m and ϕ_{II} were reduced to 0.1 or less (Fig. 4B). Although there was no statistically significant difference between stress treatment with UV-A or UV-A+B, thalli treated with UV-A+B had slightly but consistently lower quantum yields. The Q pool was 30%–60% reduced at low irradiance, increasing to nearly 100% at $380 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 4E). A greater degree of reduction was seen due to the UV-A+B test compared with the UV-A test, but the response was highly variable. The degree of recovery after the stress differed among the fluorescence parameters. Quantum yield recovered somewhat during the next 3 h in low PAR, with the tendency for lesser recovery when the stress test contained UV-B (Fig. 4C), whereas the reduction status of the Q pool recovered completely in each case, differing only slightly from initial values at low irradiance (Fig. 4F).

Response to a standard UVR stress after acclimation to spectral conditions and MAA synthesis. The response of thalli to the 3-h stress test was measured again at the end of the 40-day acclimation and MAA synthesis period. Results of measurements performed before, after the 3-h treatment, and after the 3-h recovery are presented in Figures 5–7. Complete fluorescence versus irradiance curves were measured in each case, but only the results for F_v/F_m (Fig. 5), ϕ_{II} (Fig. 6), and Q pool reduction status (Fig. 7) at the low and high irradiance endpoints (7 and $380 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) are shown for clarity. In almost every case, there was a significant effect (usually $P < 0.001$) of acclimation filter color on the results of the stress test, as indicated in the figures.

The ratio F_v/F_m of thalli acclimated to white or blue light declined significantly less after the 3-h exposure to UV-A or UV-A+B compared with that of green- or red-acclimated samples (Fig. 5) and less than observed initially (cf. Fig. 4A). Recovery was also significantly greater, especially after UV-A exposure, indicating increased capacity to cope with UVR stress. In contrast, there was no difference in response between red-acclimated samples and initial thalli. Thus, thalli with the higher amount of MAAs had a higher optimal quantum yield under these circumstances. However, samples from the white and blue treatments differed in their response to UV-A, even though they contained similar amounts of MAAs. “White” thalli maintained a significantly higher optimal quantum yield than those from the blue treatment after exposure to the 3-h UV-A, but this difference disappeared when UV-B was included in the test radiation, and the F_v/F_m of all samples was lower. Differences among treatments at the start of the test, although statistically significant, were quite small relative to changes due to the stress and therefore were probably not biologically significant.

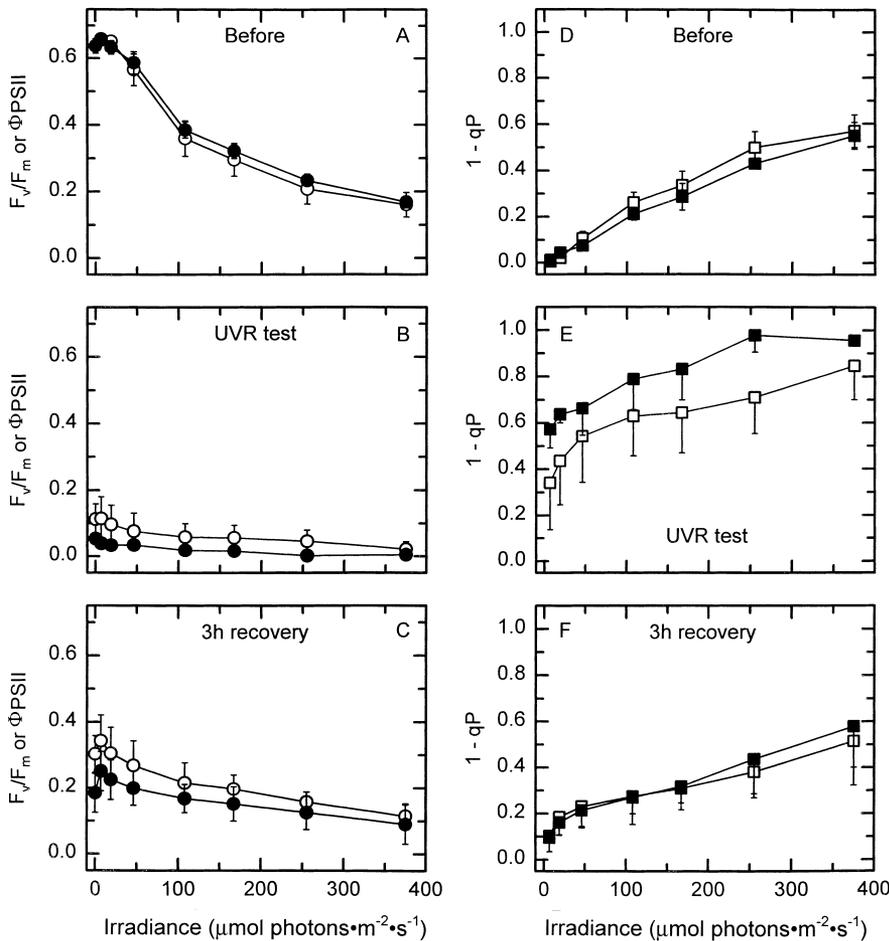


FIG. 4. Fluorescence versus light response curves of *Chondrus crispus* thalli before growth under white, blue, green, or red filters. Measurements were made before and after a 3-h test exposure to UV-A (open symbols) or UV-B (filled symbols) and after 3-h recovery in PAR. Unweighted test irradiance: 1.4 W·m⁻² UV-B (300–320 nm), 20 W·m⁻² UV-A (320–400 nm), and 20 μmol photons·m⁻²·s⁻¹ PAR (400–700 nm). Weighted UV-B (PAS₃₀₀): 0.139 W·m⁻². For the UV-A only treatment, UV-B lamp output was blocked using a Folex filter. Recovery occurred in 20 μmol photons·m⁻²·s⁻¹ PAR. (A–C) Changes in the optimal quantum yield, F_v/F_m , after a 5-min period of darkness and then changes in Φ_{II} in response to PAR between 7 and 380 μmol photons·m⁻²·s⁻¹. (D–F) Changes in the reduction status of the Q pool. Means \pm SD, $n = 4$.

When measured at 7 μmol photons·m⁻²·s⁻¹, Φ_{II} of samples acclimated to white or blue light was also higher after the UVR test than that of thalli acclimated to green or red light (Fig. 6, A and B). Again, testing with UV-A lowered Φ_{II} of thalli from the blue treatment more than those from the white treatment, despite equal amounts of MAAs. Again, this difference disappeared when UV-B was present. There was no significant difference between green and red samples in either case. After the 3-h recovery from the UV-A test, Φ_{II} was close to the starting values in the case of white and blue samples but significantly lower in the case of green and red samples (Fig. 6A). Recovery from the UV-A+B test was greater in white samples than in blue, green, or red samples (Fig. 6B). Despite the fact that red light-acclimated thalli had little accumulation of MAAs and performed worst among the treatments under UVR stress, these samples did show some signs of increased capacity to cope with UV, as recovery was greater than that seen initially (cf. Fig. 4C).

When measured at 380 μmol photons·m⁻²·s⁻¹, the UV-A test (Fig. 6C) did not further reduce Φ_{II} of white samples or blue samples. Again, samples from the red treatment had significantly lower Φ_{II} , and green samples

were intermediate. The UV-A+B test (Fig. 6D) reduced Φ_{II} in all cases, but this time blue, green, and red samples were equally affected despite differing amounts of MAAs. After the recovery period, Φ_{II} was highest in white and blue (UV-A) or just white thalli (UV-A+B).

Before applying the UVR test, the quinone pool was highly oxidized at 7 μmol photons·m⁻²·s⁻¹ (Fig. 7, A and B) and approximately 50% reduced at 380 μmol photons·m⁻²·s⁻¹ (Fig. 7, C and D). No clear trends or correlations with MAA content were observed. After the test, a slightly higher proportion of Q was reduced at low light in green- and red-acclimated thalli, but after recovery there was no difference among treatments. At high light, the UV-A test had no effect on samples grown in white, blue, or green light, but almost 100% of Q was reduced in red-treated thalli. In contrast, the UV-A+B test led to 100% reduction in blue- and green-acclimated thalli as well. Again, inclusion of UV-B separated the response of white and blue samples, despite equal MAA content. White light-acclimated samples recovered fully after either test. In contrast, thalli from the blue treatment recovered to a similar extent after the UV-A test but less so when the test contained UV-B. The least recovery was found in red samples. In summary, those thalli with more MAAs

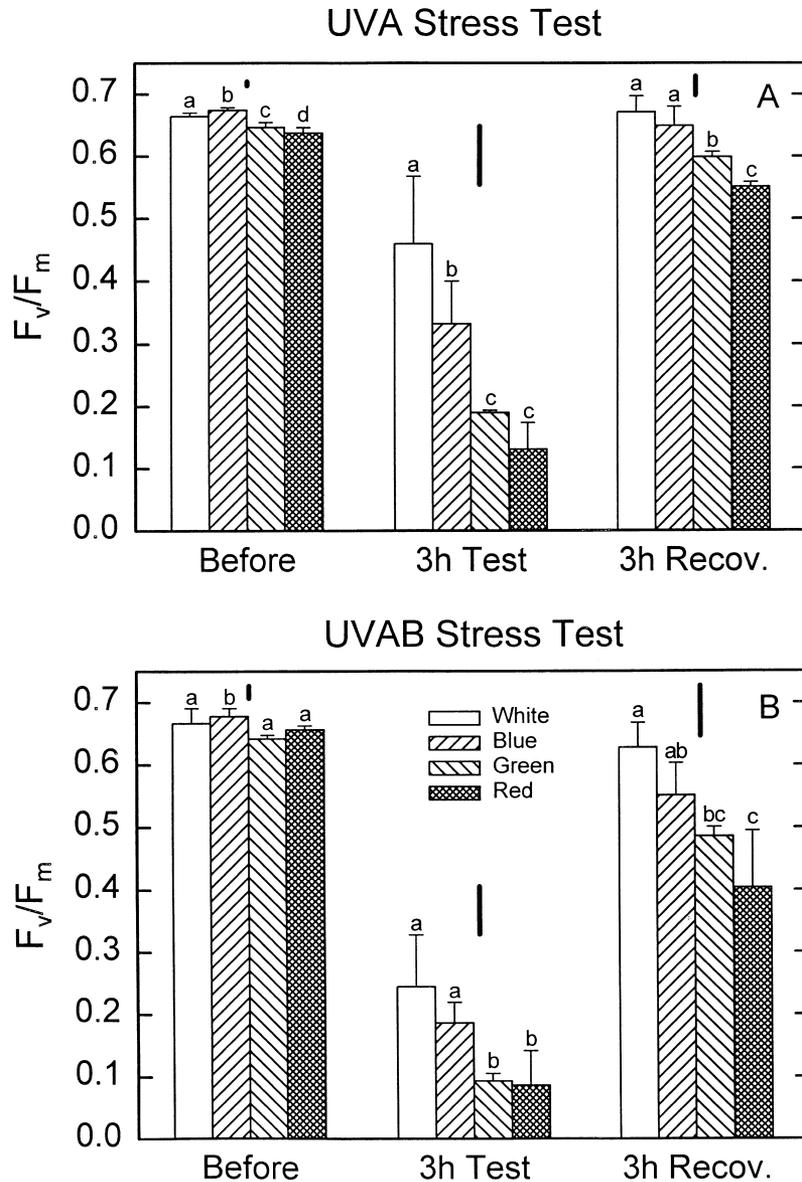


FIG. 5. Comparison of F_v/F_m of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. Changes are due to exposure to the laboratory UV-A (A) or UV-A+B (B) stress tests and during subsequent recovery. Test and recovery conditions as in Figure 4. Means \pm SD, $n = 4$; heavy lines above each group of bars are the least significant difference.

usually had high photosynthetic performance under UVR stress, but those with equally high MAA content (white and blue) differed with respect to relative protection from UV-A or UV-A+B.

Effect of prior exposure to blue light on UVR-induced MAA synthesis. Neither fresh subtidal *C. crispus* (Fig. 8, A–C, “0” days) nor samples that had already been exposed to the 2-week pretreatment in blue light (Fig. 8, D–F, “0” days) contained any detectable MAAs. This was in contrast to results from the earlier experiments (Fig. 3) but was likely due to the reduced global irradiance at this later date. MAA synthesis in *C. crispus* has been shown previously to be dose dependent (Karsten et al. 1998a). During the next 2 weeks, a small increase in the level of palythine was observed in samples in blue light, occurring faster if there had been previous blue light exposure (Fig. 8, A and D)

but to the same extent at the end of the experiment. Total MAA content was lower than in the first experiment, in agreement with the lower global irradiance at that time of year. In contrast, the amount of shinorine present in both field and blue-pretreated samples rose significantly within 3 days of transfer to UV-A or UV-A+B (Fig. 8, B, C, E, and F). However, the sequential application of blue light and UVR led to a 10-fold greater initial synthesis of shinorine than any treatment alone, a more than additive effect of either blue or UV-A or UV-A+B (note different scales in E and F). The presence of UV-B in the spectrum made little difference in the levels of MAAs measured but might have contributed to a decline in MAA content at the end of 2 weeks. Samples that had not been previously treated in blue light also contained a significant amount of an unidentified UV-absorbing compound (Fig. 8, B

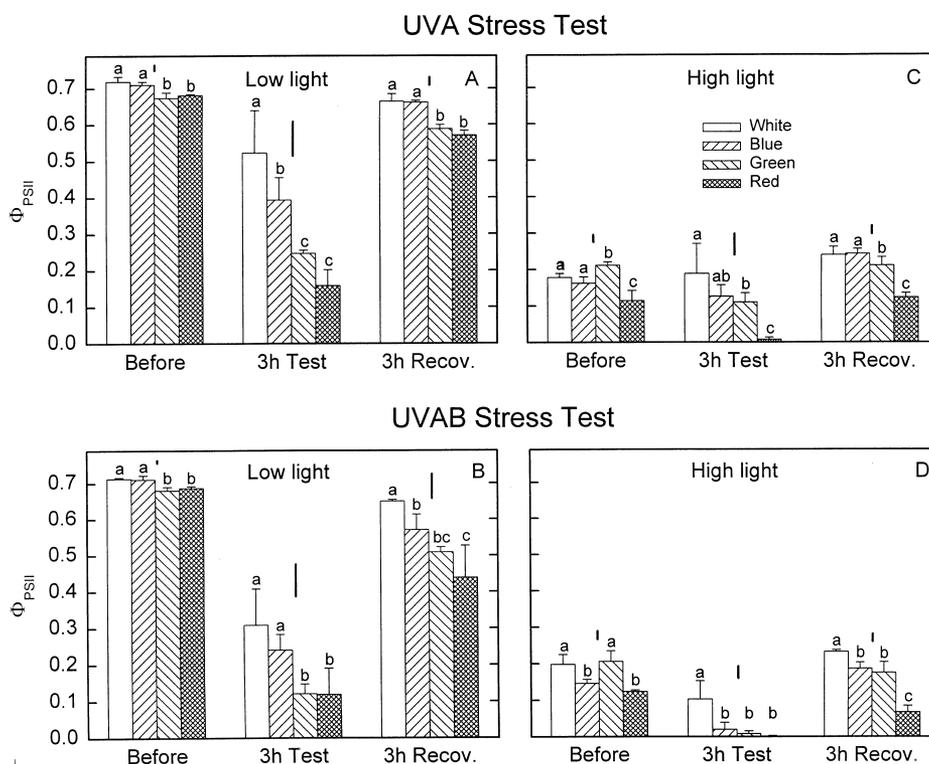


FIG. 6. Comparison of Φ_{PSII} of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. (A and B) Differences among treatments when measured at low light ($7 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). (C and D) Differences among treatments when measured at high light ($380 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). (A and C) Changes due to exposure to the laboratory UV-A stress test and subsequent recovery. (B and D) Changes due to exposure to the laboratory UV-A+B stress test and subsequent recovery. Test and recovery conditions as in Figure 4. Means \pm SD, $n = 4$. Different letters designate significantly different treatments; heavy lines above each group of bars are the least significant difference.

and C), but total UV-absorbing compounds were still much less than observed in blue-pretreated samples.

DISCUSSION

MAA synthesis and protection of photosynthesis from UV stress. Natural UV-B radiation clearly has the potential to inhibit photosynthesis of many macroalgae *in situ* (e.g. Häder et al. 1996, Sagert et al. 1997) and especially when thalli are moved from low radiation environments at depth to near-surface levels of UV-B (e.g. Wood 1987, 1989, Larkum and Wood 1993, Herrmann et al. 1995, Hanelt et al. 1997). For species occurring over a wide range of depths, the degree of inhibition is usually markedly less in samples from shallow water (Dring et al. 1996, Bischof et al. 1998). There are a number of possible photosynthetic targets for damage by UV-B, including RUBISCO (Nogués and Baker 1995, Lesser 1996) and the donor and/or acceptor side of PSII reaction centers (Post et al. 1996, Vass et al. 1999). The widespread occurrence of MAAs in organisms growing in high irradiance and the broad UV absorption spectrum support the hypothesis that MAAs act as sunscreens in macroalgae. Most evidence relating MAAs to reduced UV sensitivity are correlative, but in the dinoflagellate *Gymnodinium sanguineum*, MAAs specifically reduced the action

spectrum of damage to photosynthesis in UV wavelengths (Neale et al. 1998). In contrast, there are also cases where accumulation of MAAs or unidentified UV absorbing compounds offered only limited protection of photosynthesis, pigment content, or growth rate of macroalgae (Wood 1989, Lesser 1996, Franklin et al. 1999).

Because the photosynthetic efficiency of lab-cultured *C. crispus* was equally reduced by UV-A and UV-A+B and the combination of MAAs in *C. crispus* results in a broad UV absorption spectrum, we were interested in whether there was a differential change in the MAA content of thalli under different light treatments and whether there was differential UVR sensitivity based on the accumulation of MAAs. In agreement with other studies, the photosynthetic efficiency of thalli with higher MAA content was generally more resistant to UVR, either when the comparison was made to thalli sampled before the growth experiment (Fig. 4) or among the treatments after the experiment (Figs. 5 and 6). In those treatments where MAAs were synthesized, the differences in the results of the UV-A tests before and after MAA synthesis were much greater than the differences in the results of the UV-A+B test. This was unexpected because the major compound palythine has an absorption maximum of 320 nm. But

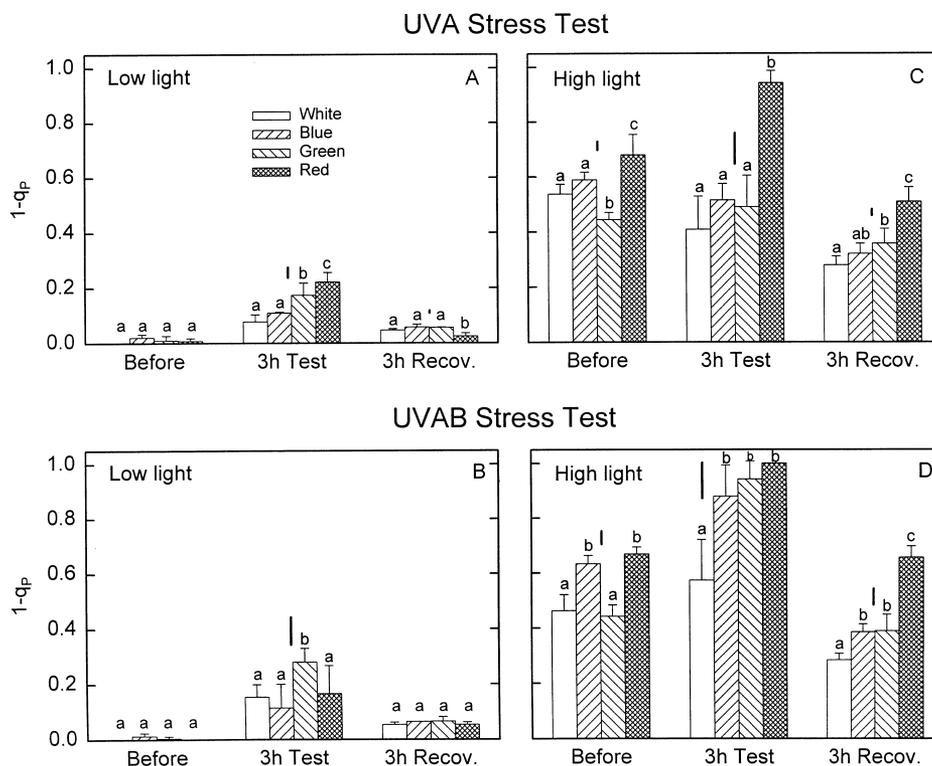


FIG. 7. Comparison of the reduction status of the Q pool of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. A–D as in Figure 6. Test and recovery conditions as in Figure 4. Means \pm SD, $n = 4$. Different letters designate significantly different treatments; heavy lines above each group of bars are least significant differences where significant differences were detected.

the transfer to higher irradiance in general appeared to have a positive effect on the response to UV. Even without substantial MAA synthesis, red and green light-treated samples recovered to a greater degree after the UV stress than observed initially.

Additionally, there was often a significant difference between samples with equal MAA content (white and blue) in their change in photosynthetic efficiency after UV-A versus UV-A+B stress. Blue light-grown thalli were usually significantly more sensitive to UV-A than were thalli grown in white light but equally susceptible to the more damaging combination of UV-A+B. This may have reflected a greater capacity of white light-grown samples for damage repair or UV-A screening by means other than MAAs. Or, it may simply be an artifact arising from the fact that white light thalli contained lower amounts of phycoerythrin and phycocyanin relative to chl *a* than blue light samples and therefore had smaller photosynthetic unit targets. On the other hand, UV-B stress continued to have a significant effect on the reduction status of Q that was independent of MAA content and the ratio of phycobiliprotein/chl *a*. After the UV-A+B stress, Q pools in blue-treated samples were as highly reduced at light saturation as in green and red samples. One interpretation of these results is that exposure of blue-, green-, or red-treated thalli to UV-B disrupted electron flow beyond the plastoquinone pool, perhaps through

damage to ATP synthase or RUBISCO, but that growth in white light somehow reduced this effect independently of MAA content. A highly reduced Q pool and high degree of thylakoid membrane energization would have the additional effect of increasing the likelihood of photoinhibition by PAR and damage to PSII (Osmond et al. 1993).

It is quite likely that growth under the three different spectra led to a number of additional metabolic changes besides MAA synthesis. For example, blue and red light are well known to favor synthesis of proteins and carbohydrates, respectively (Kowallik 1987). Therefore, we hesitate to draw any specific conclusions as to exact mechanism by which the presence of MAAs in white versus blue light-grown thalli is correlated with improved photosynthetic performance after UV-A or UV-A+B treatment.

Induction of MAA synthesis in Chondrus crispus. In a number of algae, the synthesis of MAAs has been shown to be induced either by UV-B, UV-A, or PAR, or a combination of these wavelengths (Carreto et al. 1990, Riegger and Robinson 1997, Karsten et al. 1998a). In other species, MAAs are constitutively expressed for generations under laboratory culture conditions (Jeffrey et al. 1999) or are apparently uninducible (Hoyer et al. 2001). This variation of cause and effect makes it difficult to come to a consensus about particular triggering mechanisms. Furthermore, the final

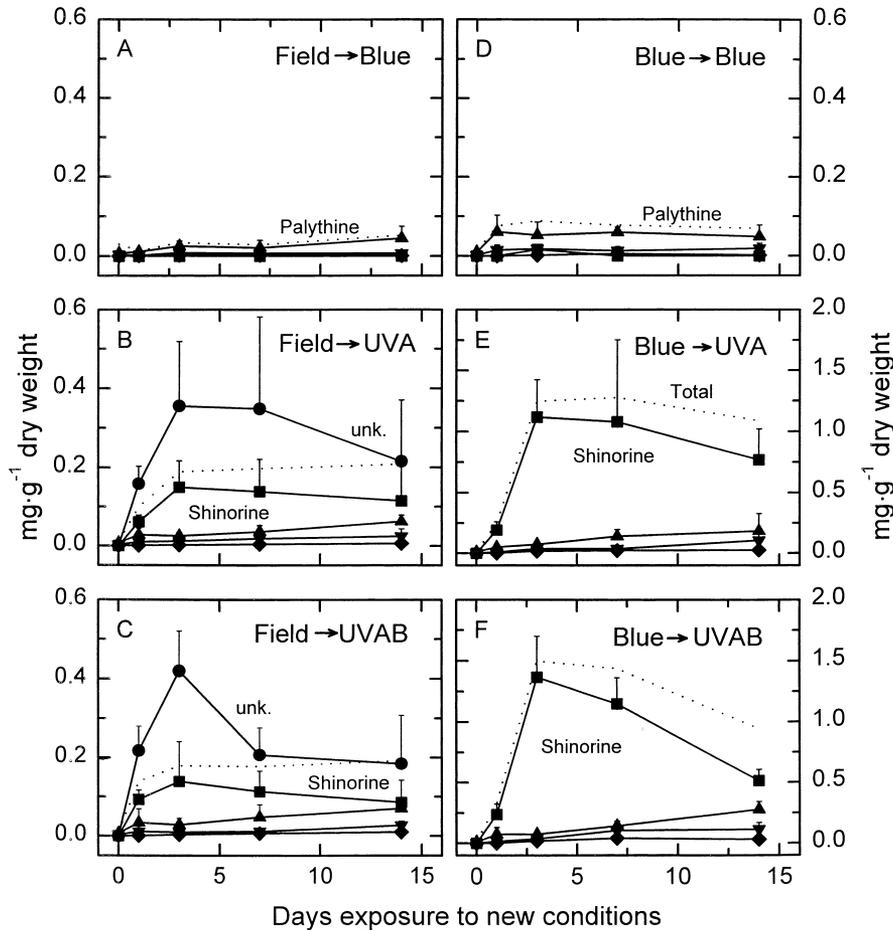


FIG. 8. A comparison of the time course of the accumulation of MAAs in *Chondrus crispus* collected directly from 6 m (A–C) or preexposed to blue light (D–F). The two types of thalli were treated as follows for the induction of MAAs: (A and D) 2 weeks exposure under blue light, (B and E) 2 weeks exposure under UV-A (no PAR, UG5 + Folex filter), and (C and F) 2 weeks exposure under UV-A+B (no PAR, UG5 + Ultraphan 290 filter). Amounts are expressed on a thallus dry weight basis. Please note the different scale in E and F. The major MAAs are identified on the panels; only trace amounts of asterina-330 and palythene were detected. The dotted line is the sum of all identified MAAs. Means \pm SD, $n = 5$.

concentrations of MAAs in an organism, and possibly the particular MAA composition, reflects the quantity of radiation applied (Carreto et al. 1990, Riegger and Robinson 1997, Karsten et al. 1998a, Franklin et al. 1999). Part of the difficulty in interpretation comes from the fact that in a number of cases, an increase in MAA concentration from some previous particularly low amount is reported. Thus, it is difficult to distinguish between signals for synthesis and control of signal transduction pathways that have already been initiated. It is possible that more than one photoreceptor or signal transduction pathway might be involved in the overall process leading to high MAA concentrations, analogous to interactions among various photoreceptors that have been reported in higher plants (Casal 2000).

In the present experiments, evidence has been found for specific blue and UV-A radiation-mediated MAA synthesis from conditions where no MAAs were present and for synergism between the two responses

with respect to synthesis of a single MAA, shinorine. Synthesis of palythine, the principle MAA found in eulittoral populations of *C. crispus*, was clearly induced by blue light. The limited palythine synthesis observed in green light may have been due to the spectral overlap of the filters in the 450- to 550-nm region. Elimination of wavelengths less than 380 nm from the white light treatment dramatically reduced the shinorine synthesis seen previously in PAR (Franklin et al. 1999), and no shinorine was detected under blue light. In contrast, shinorine synthesis was specifically induced by exposure of samples to UV-A radiation, confirming our earlier results with this species (Karsten et al. 1998a). Shinorine is by far the most common of MAAs reported in macroalgae so far, in species collected from tropical to Arctic waters (Banaszak et al. 1998, Karsten et al. 1998b), and was the most rapidly accumulated compound synthesized in *Stylophora* colonies exposed to UVR under controlled laboratory conditions (Shick et al. 1999). Wavelength

dependence for MAA synthesis in some species of microalgae has been shown previously. In particular, Carreto et al. (1990) found that growth in blue light increased the total UV absorbance of *Alexandrium excavatum* cells, but not to the same degree as white light. A species-specific effect was reported by Riegger and Robinson (1997), with MAA synthesis in Antarctic diatoms responding maximally to wavelengths between 370 nm and 460 nm but not to UV-B and in the prymnesiophyte *Phaeocystis antarctica*, maximally to 340 nm, down to 305 nm.

Shick et al. (1999) used the inhibitor glyphosate to demonstrate that synthesis of 10 MAAs, including shinorine and palythine, proceeds along a portion of the shikimate pathway, as had been inferred from the work of Favre-Bonvin et al. (1987) on fungal mycosporine. With more than 20% of fixed carbon passing along this route (Herrmann 1995), the shikimate pathway is the process by which the aromatic amino acids phenylalanine, tyrosine, and tryptophan and a number of precursors for secondary metabolites are made in microorganisms and plants. In particular, phenylalanine acts as a precursor for the synthesis of UV-absorbing flavinoids. Recent evidence for the complex photoregulation of flavin synthesis in *Arabidopsis* appears relevant to the discussion of regulation of MAA synthesis in *C. crispus* and perhaps in other species. The first step of the flavinoid pathway is catalyzed by chalcone synthase (CHS). Christie and Jenkins (1996) and Fuglevand et al. (1996) demonstrated that UV-B and UV-A/blue light induced CHS expression by separate pathways, only the latter of which involved the cryptochrome CRY1 photoreceptor. In addition, UV-A acted synergistically with UV-B to generate a transient signal for stimulating the CHS gene promoter, whereas blue light acted synergistically with UV-B by a stable signal. These data were interpreted as representing separate pathways for signal transduction in the flavinoid pathway.

The details of the pathway responsible for synthesis of specific MAAs and its relationship to specific steps in the shikimate pathway remains to be elucidated. However, support for the hypothesis that two photoreceptors or signal transduction pathways account for MAA synthesis in *C. crispus* comes from the observation that maximal shinorine synthesis occurred when blue light preceded the UV-A treatments, to quantities greater than those predicted from an additive response. The advantage of such a signal transduction system for a marine organism would lie in the preferential attenuation of UVR in the water column by dissolved organic material, or gelbstoff, with a characteristic absorption spectrum that increases exponentially at decreasing UV wavelength. Organisms able to sense a change in the amount of blue light present and respond by directing more carbon skeletons toward the synthesis of UV-absorbing compounds would have an advantage if the change in blue light presaged an increase in UV, as seen on a seasonal basis at high latitudes. In a climate change scenario, the depletion of

stratospheric O₃ only leads to an increase in the shortest UV-B wavelengths; thus, at first consideration this triggering system would not be of particular advantage during periods of enhanced UV-B. However, UV-B has been shown to directly affect water column clarity by the photooxidation of gelbstoff (Morris and Hargreaves 1997). In this case, the gradual progression to ever shorter UVR as gelbstoff was oxidized would lead to greater inhibition of photosynthesis (Arrigo and Brown 1996) unless a photoprotective response was triggered before the shortest UV wavelengths were transmitted.

We did not measure a strict dose-response in these experiments, but a rough calculation suggests that in *C. crispus*, the blue light receptor saturates at irradiances that are at most 30% of incident (Table 1). Neither have we identified a specific photoreceptor molecule, though detailed action spectra for MAA synthesis in *C. crispus* is underway (G. Kräbs and U. Karsten, Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany, unpublished data). It is also interesting to note that based on the difference in pigment concentration between white and blue light-grown samples (Table 3) and the amount of total radiation received (Table 1), the amount of PUR received by the thallus appeared to have no direct effect on MAA synthesis in the short term but affected the rate of growth. It is clear that there are many more parts to the MAA induction puzzle to be clarified, for example, the induction of mycosporine-glycine synthesis by osmotic stress in darkness (Portwich and Garcia-Pichel 1999) and the basis by which macroalgae can be divided into those that do not ever appear to have MAAs, those in which MAAs can be induced, and those that constitutively contain them (Hoyer et al. 2001).

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