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Release of ultraviolet-absorbing compounds by the red-tide dinoflagellate *Lingulodinium polyedra*

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Abstract We tested the hypothesis that ultraviolet-absorbing compounds known as mycosporine-like amino acids (MAAs) are not only synthesized but also excreted by marine phytoplankton. An experiment was performed with cultures of the marine dinoflagellate *Lingulodinium polyedra* (previously known as *Gonyaulax polyedra*) exposed to visible (photosynthetically available, PAR, 400 to 700 nm) and ultraviolet (UV, 290 to 400 nm) radiation. Absorption properties of both particulate and dissolved organic matter pools (POM and DOM, respectively) showed maxima in ultraviolet absorption at 360 nm. Chromatographic analysis confirmed the presence of MAAs in both pools. Release of organic matter by *L. polyedra*, as measured spectrophotometrically by changes in UV absorption in the surrounding medium, showed a differential increase at 360 nm in cultures exposed to UV-B + PAR radiation. The changes in absorption in the DOM fraction were inversely proportional to intracellular UV absorption. Photodegradation experiments in which the DOM fraction was exposed to visible and UV-B radiation showed a decrease in absorption with dose. First-order photooxidation decay rates varied between -0.005 and $-0.26\ m^2$ (mol quanta)$^{-1}$ and were also a function of the initial optical density (OD). These results indicate that UV-absorbing compounds synthesized by phytoplankton, such as certain dinoflagellates, may be a component of the DOM pool in surface waters of the ocean and contribute to the attenuation of UV radiation in the water column. Photooxidation consumes only 3 to 10% of the daily production of the DOM absorbing between 280 and 390 nm (including MAAs). This suggests that MAAs dissolved in seawater may contribute to the decrease of UV transmission through the water column on a time scale representative of phytoplankton growth (days) and bloom development (weeks).

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Introduction

Natural ultraviolet (UV) radiation may have detectable effects on organisms to depths of 10 to 15 m (Lorenzen 1979; Hunter et al. 1979; Smith et al. 1992). The responses induced by UV radiation can be expected to vary from short-term reversible effects to long-term irreversible effects. Reduction in photosynthetic rates (Maske 1984; Helbling et al. 1992; Smith et al. 1992) and increased fluorescence quenching are observed first, followed by pigment bleaching (Döhler 1984, 1985; Miller and Carpentier 1991), retardation of cell division, and cell death (Karentz et al. 1991a). The effect on reduced viability and ability of the cells to divide presumably is due to interactions between DNA and UV radiation (Karentz et al. 1991a).

Effects of UV radiation on cell metabolism and viability depend on phytoplankton taxa. Worrest et al. (1981) documented transitions in the taxonomic assemblage of estuarine mesocosms after exposure to UV. A model to describe these transitions required that the different taxa have differential sensitivities to UV-B exposure. This effect is presumed to be in part due to the ability of certain species to adapt and minimize the effects of UV radiation, including DNA repair (Karentz et al. 1991a), increase in fluorescence yield (Demmig-Adams et al. 1990), and the synthesis of intracellular UV-absorbing compounds thought to act as screens of harmful radiation (Jokiel and York 1982; Siebeck 1988).

The mycosporine-like amino acids (MAAs; Favre-Bovin et al. 1976) synthesized by marine algae absorb in the UV region of the spectrum between 300 and 370 nm. High UV absorption was first observed in a cyanobacteria, *Oscillatoria* sp., by Shibata (1969).

Later, Yentsch and Yentsch (1982) observed high UV absorption in marine dinoflagellates. Chromatographic analysis has identified these compounds as MAAs in dinoflagellates (Carreto et al. 1990a), diatoms (Karentz et al. 1991b), and cyanobacteria (Miller and Carpentier 1991). Based on patterns of UV absorption and underwater distribution of UV radiation, several studies have suggested that MAAs act as natural screens to UV radiation and as such should be considered photoprotective (Dunlap et al. 1986; Vernet et al. 1994). Comparison between absorption and photosynthetic action spectra of the dinoflagellates *Lingulodinium polyedra* and *Prorocentrum micans* suggest that the energy absorbed in the UV is not utilized for photosynthesis (Vernet et al. 1989: *L. polyedra* referred to as *Gonyaulax polyedra* therein).

Release of organic matter by phytoplankton is considered to be a major source of dissolved organic matter (DOM) in the ocean (Lee and Henrichs 1993). Absorption of light by DOM in the ocean increases exponentially from the visible towards the UV region of the spectrum. Recent studies of the composition of DOM suggest that polysaccharides contribute up to 50% of the total pool (Benner et al. 1992), a large part of which is assumed to be released by phytoplankton. The composition of DOM also seems to be influenced by the dominant phytoplankton taxa (Sundh 1992; Ridal and Moore 1993). In lakes dominated by dinoflagellates, the average composition of low molecular weight components (< 1000 daltons) was 5 to 33% amino acids, 23 to 57% organic acids and 21 to 53% carbohydrates.

In this study, we tested the hypothesis that MAAs, synthesized by the dinoflagellate *Lingulodinium polyedra* as possible screens for UV radiation, are released by cells during exposure to UV-B radiation and absorb UV light in the water column, thus becoming a potential mechanism to decrease UV transmission in the water column. We present experiments to estimate the release of MAAs by *L. polyedra* cultures exposed to UV-B + photosynthetically available radiation (PAR) and estimates of photooxidation rates under UV − B + PAR radiation of MAAs dissolved in seawater.

Materials and methods

The inoculum for *Lingulodinium polyedra* cultures (Dodge 1989), supplied by Dr. W. Thomas, is of unknown origin, but most likely from local coastal waters. Axenic cultures were maintained in *Gonyaulax polyedra* medium (GPM: Loeblich 1975) under a saturating PAR irradiance of 120 µmol quanta $m^{-2} s^{-1}$ as measured at 24 °C with a Biospherical Instruments Inc. QSL-100 PAR meter. For experimentation, cultures used were in log-phase growth ($\mu \sim 0.3\ d^{-1}$), as determined by changes in in vivo fluorescence.

Experiments were conducted in an incubator containing both PAR and UV-B sources. The lamps were housed in a wooden box, $1.22 \times 0.91 \times 0.61$ m, and painted black with flat, non-toxic, non-reflective paint. An air-duct booster was mounted on one end of the box and an exhaust vent was installed on the other end; the airflow maintained a constant temperature of 24 °C. PAR radiation was provided by two 1.22 m GE cool-white lamps and UV-B radiation by a Westinghouse FS-40 sunlamp. The UV-B lamp was covered with Cellulose III acetate to filter radiation below 290 nm (UV-C). UV-A irradiance originated from both UV-B and PAR lamps (Fig. 1a).

UV and PAR lamps were calibrated with an Optronics Laboratory Inc. Spectroradiometer Model 752. Emission spectra for UV-B and PAR lamps displayed peaks at 313 and 580 nm, respectively (Fig. 1). The spectrum for the UV-B lamp was taken at single nanometer intervals between 270 and 350 nm. For the PAR lamp the spectra were taken at 10 nm intervals and integrated over that same interval from 260 to 800 nm.

During experiments, *Lingulodinium polyedra* cultures were placed in 120 ml cylindrical quartz vessels (30 cm in length, 2.5 cm in diam), exposed to a UV-B source at 0.127 W m^{-2} or 0.53 µmol quanta $m^{-2} s^{-1}$ (1 W m^{-2} = 4.17 µmol quanta $m^{-2} s^{-1}$: Morel and Smith 1974) and a PAR source of 30.9 µmol quanta $m^{-2} s^{-1}$. The ratio of UV-B to PAR was 0.017, approximately two to three times the expected irradiance at noon on a summer day off Scripps Pier at La Jolla, California, USA (32 °N). A control tube was placed beneath a Plexiglas shield (UF-3) with 50% transmission at 390 nm (Jokiel and York 1982), and exposed to the same conditions as the experimental vessels. Significant differences between treatments were evaluated with non-parametric statistics (Zar 1984).

The optical thickness of the experimental vessels at all wavelengths was examined so that the photon flux absorbed would impose no limitations on exudation or photooxidation rates (Dister and Zafiriou 1993). Optical thickness at 313 nm was calculated by Lambert Beer law:

$$I = I_0 \exp^{-K_D * Z}, \quad (1)$$

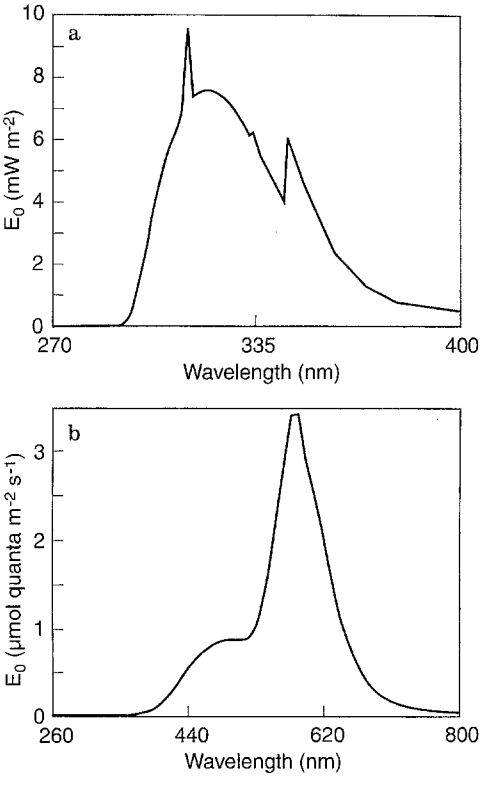

Fig. 1 Spectral irradiance (E_0) of lamps used in all experiments. **a** Westinghouse FS-40 Sunlamp with maximum emission at 313; **b** GE cool-white PAR lamp with emission peak at 580 nm

where I = irradiance (mW m^{-2}), I_0 = incident irradiance (mW m^{-2}), z = depth (m), and K_D = ratio between absorption coefficient [$a(\lambda)$, m^{-1}] and mean cosine of the light path (μ) through the vessel and medium (Stavn and Weidemann 1988). The absorption coefficient is composed of three parts: absorption by the particulate (POM), and soluble components (DOM), and the water itself (Mitchell 1992). POM, DOM + water absorption values were obtained spectrophotometrically for all experiments.

The mean cosine was estimated based on observed field trends and Monte Carlo simulations, since no values for $\bar{\mu}$ have been published for the UV region of the spectrum at 313 nm or for UV sunlamps. The evidence used was: (1) In natural ocean waters $\bar{\mu}$ decreases with depth as the light becomes more diffuse (Stavn and Weidemann 1988. High values of $\bar{\mu}$ have been modeled in the Sargasso Sea, ranging from 0.90 to 0.98 for the NOARL blue water model (Stavn and Weidemann 1992) and the NORDA Monte Carlo simulation (Stavn and Weidemann 1988); the quartz experimental vessels have a depth of $z = 0.025$ m, which mimics the conditions at the water surface, and thus we expect a high $\bar{\mu}$. (2) High values of $\bar{\mu}$ have been recorded for the red region of the light spectrum (Kirk 1983, pp 124–127); because of optical similarities between red and UV radiation in the water, it would be expected that, as in the red region, UV would also have a high $\bar{\mu}$ (K. Baker, Scripps Institution of Oceanography, personal communication). (3) The zenith angle has an additional effect on $\bar{\mu}$; a Monte Carlo simulation showed a 0.15 decrease in $\bar{\mu}$ for a zenith angle-change from 0 to 45° (Kirk 1981). (4) Field data and computer models have shown that at the surface, and for a backscattering to absorption ratio $b:a < 1$, a high μ is expected. For example, in the San Diego Harbor, with $b:a = 1$, zenith angle = 0 to a depth of 0.1% of incident irradiance and an optical depth of 0, a $\bar{\mu} = 0.98$ was estimated (Bannister 1992). (5) A high μ would be expected in laboratory cultures, since the $b:a$ is < 1 (Bricaud et al. 1983). In summary, for our calculations $\bar{\mu}$ was estimated at 0.85 [1.00 (the highest possible value) − 0.15 (to account for a zenith angle = 45°)]. Based on this μ, the vessels were optically thin, absorbing < 25% of the incident light (Dister and Zafiriou 1993). Optical thickness varied from 80 to 90% transparency for exudation experiments, and from to 85 to 89% transparency for photooxidation experiments.

To estimate the rate of MAA exudation by *Lingulodinium polyedra*, cultures were irradiated with UV-B + PAR for 60 h (Experiment 1). Aliquots of 25 to 30 ml were removed from the quartz vessels every 12 h for analysis of particulate and dissolved absorption, chlorophyll *a* concentration, and microscopy analysis. Glassware for sampling had been rinsed with 10% HCl, Milli-Q water, 90% methanol, and again with Milli-Q water. Samples were concentrated by differential pressure onto combusted Whatman GF/F filters; the filtrate was collected into scintillation vials and kept in the dark at − 16 °C, while the filters with the cells were analyzed immediately. Spectrophotometric measurements of dissolved samples were conducted within 12 h of sampling. Absorption of the filtrate stored under the same condition showed a loss of < 5% after 5 d. Absorption values in Experiment 1 are based on single readings due to lack of volume for replicate sampling. From similar experiments with *L. polyedra* cultures, we have determined a coefficient of variation in absorption in replicate samples ($n = 4$) of 14% at 310 nm, 13% at 330 nm, and 13% at 360 nm.

Rates of photooxidation of MAAs dissolved in seawater were estimated by exposing filtrate from *Lingulodinium polyedra* cultures to UV-B and PAR radiation for 48 h (Experiments 2 and 3) and 8 h (Experiment 4) with the same intensities described above. Cells were removed by use of differential pressure with combusted GF/F filters, and the remaining filtrate was placed in 120 ml quartz tubes. Samples of 25 to 30-ml were removed from the vessels at known time intervals and stored as described above.

Absorption properties were measured with a Perkin Elmer Lambda 4A spectrophotometer connected to an IBM PC. Absorption by the cells or particulate organic matter (POM) was measured after concentration onto Whatman GF/F filters that were placed on quartz holders. Blank GF/F filters saturated with 0.2 μm-filtered seawater were used as a reference. Absorption (m^{-1}) was estimated following Mitchell (1990). DOM absorption in the filtrate obtained from concentrating the cells was measured in 10-cm quartz cuvettes. As reference, 0.2 μm-filtered seawater was treated with H_2O_2 (1:2500, v/v) and exposed to intense UV radiation for 12 h (Armstrong and Tibbits 1968). Independent measurements were conducted in the late Dr. P. Williams laboratory (Scripps Institution of Oceanography) to test this method; analysis of the organic-free seawater using high-temperature combustion gas-chromatography gave concentrations of < 30 μM of carbon which confirmed that the absorption of the seawater after treatment with the wet-combustion method was sufficiently low to be used as a blank. In addition, the possibility that DOM absorption in the filtrate was due to cell breakage during filtration was tested. Absorption in filtrate obtained by gravity filtration was within 1 to 2% of that obtained with < 5 psi differential pressure (data not shown).

Photooxidation rates were estimated based on total dose ($k(\lambda)$, m^2 mol^{-1}) and time of exposure ($\kappa(\lambda)$, s^{-1}) for the same experimental conditions (Kouassi and Zika 1992). First-order rate constants were estimated by linear regression:

$$k(\lambda) = 1/\Delta I \cdot \ln(Abs_t/Abs_{t0}), \quad (2)$$

where $k(\lambda)$ = photooxidation rate constant [m^2 (mol quanta)$^{-1}$], I = total dose integrated over time [(mol quanta) m^{-2}], Abs_t = absorption at time t (m^{-1}), and Abs_{t0} initial absorption (m^{-1}). The same procedure was applied for $\kappa(\lambda)$ (s^{-1}) where the rate constant was estimated over the time of exposure.

In vivo fluorescence measurements were performed in a Turner-Designs fluorometer. Chlorophyll *a* concentration was estimated on a 90% acetone extract of cells that had been concentrated onto a GF/F filter (Holm-Hansen et al. 1965). Extracts were kept for 24 h in the dark at 3 °C before analysis.

For high-performance liquid chromatography (HPLC) analysis, samples of *Lingulodinium polyedra* were filtered through Whatman GF/F filters which were subsequently extracted in 100% MeOH for 24 hours at 3°C. Sample extracts were clarified by filtration and injected onto the column without further treatment. To obtain extracts from the dissolved fraction, samples were filtered through a Whatman GF/F and a pre-rinsed Nuclepore 0.2 μm filter and concentrated into C-18 pre-columns. Retained DOM was eluted with three aliquots of 100% MeOH for a total elution volume of 3 ml. The first aliquot contained a mixture of seawater and methanol. The following two aliquots were solely methanol, and spectrophotometric analysis showed the MAAs to be in the second milliliter of elution. The second methanol aliquot was analyzed by HPLC without further treatment.

Extracts from both particulate and dissolved fractions were eluted with 55:45:0.1 methanol:water:acetic acid in a Brownlee C-8 column at a flow rate of 0.8 ml min^{-1} (Dunlap et al. 1986). The column was re-equilibrated between samples with 10 min of 80:20 MeOH:water. The separation was carried out in a Perkin Elmer system with detection at 315 and 340 nm.

Individual peaks were identified by retention time and absorption properties measured with a Perkin-Elmer LC-235 diode array detector. Standards used were from *Cudise racovitae*, *Palmaria decipiens*, and a subtidal anthozoan (anemone #1 as in Karentz et al. 1991b): mycosporine-glycine, retention time, $Rt = 3.03$ min, maximum absorption, $Abs_{max} = 308$ nm; shinorine, $Rt = 4.63 \pm 0.08$ min, $Abs_{max} = 334$ nm; porphyra-334, $Rt = 4.96 \pm 0.23$ min, $Abs_{max} = 334$ nm; mycosporine:glycine:valine, $Rt = 7.33 \pm 0.12$ min, $Abs_{max} = 336$ nm; asterina-330, $Rt = 7.95$ min, $Abs_{max} = 330$ nm; palythine, $Rt = 8.38 \pm 0.31$ min, $Abs_{max} = 322$ nm; palythinol, $Rt = 13.84$ min, $Abs_{max} = 332$ nm; palythene, $Rt = 14.44$ min, $Abs_{max} = 362$ nm.

Results

Absorption in the UV range (300 to 400 nm) in *Lingulodinium polyedra* cultures is higher than in the

visible range (390 to 700 nm), for both cells and filtrate (Fig. 2). Three weeks after inoculation, > 66% of the observed UV absorption in the filtrate originated from the *L. polyedra* cells. The other 33% was measured in the original GPM medium (Fig. 2, dashed line). Particulate absorption in the visible range is mainly due to the presence of chlorophylls *a* and c_2, and the carotenoids peridinin, diadinoxanthin and β-carotene (Vernet et al. 1989). In the ultraviolet region, cellular absorption is due in part to the presence of mycosporine-like amino acids:porphyra-334, mycosporine-glycine:valine, palythine, palythinol, palythene, and an unknown with Abs_{max} = 320 nm (Peaks 2 to 7 respectively, Fig. 3).

Absorption in the filtrate also presented a maximum at 362 ± 2 nm. The resemblance in UV absorption in both fractions suggested similar composition in the carbon pool exuded by *Lingulodinium polyedra*

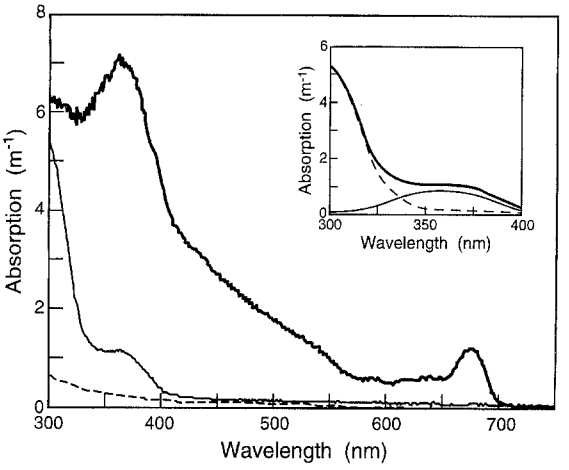

Fig. 2 *Lingulodinium polyedra.* UV and visible absorption spectra: absorption by cells in exponential growth or particulate organic matter (POM, *thick line*) and by filtrate or dissolved organic matter (DOM, *thin line*) of cultures after 24 h UV-B irradiation (*dashed line* background absorption observed in *Gonyaulax polyedra* medium before inoculation). **Insert** shoulder at 360 nm in absorption spectrum of DOM (*thick line*). mimics absorption spectrum of palythene extracted from *L. polyedra* (*thin line*) (*dashed line* difference between the two curves, is similar to absorption of initial culture medium between 325 and 400 nm)

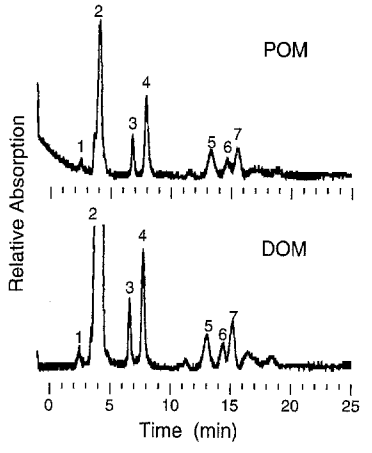

Fig. 3 *Lingulodinium polyedra.* Chromatographic separation of mycosporine-like amino acid extracted from cells (*POM*) and from their medium (*DOM*) analyzed with reverse-phase C-8 column; spectrophotometric detector set at 315 nm. Peak identification based on absorption spectrum (Abs_{max}), retention time (*Rt*), and comparison with secondary standards is *1*: solvent front, *Rt* = 3.43; *2*: porphyra-334, *Rt* = 4.96 min, Abs_{max} = 335 nm; *3*: mycosporine-glycine:valine, *Rt* = 7.42 nm, Abs_{max} = 336 nm; *4*: palythine, *Rt* = 8.48 min, Abs_{max} = 321 nm; *5*: palythinol, *Rt* = 13.63 min, Abs_{max} = 331 nm; *6*: palythene, Rt = 14.95 min, Abs_{max} = 364 nm; *7*: unknown, Rt = 15.66, Abs_{max} = 320 nm

Table 1 *Lingulodinium polyedra.* Time-series of absorption (m^{-1}) in cultures exposed to UV radiation (UV-B + PAR): Experiment 1 (*POM* particulate organic matter; *DOM* dissolved organic matter; *PAR* photosynthetically active radiation) Average coefficient of variation in absorption in replicate samples for *L. polyedra* cultures was 13% for all wavelengths measured. Increase in absorption under UV + PAR in dissolved fraction was significantly higher than control (PAR) at 360 nm (Mann–Whitney *U*-test, $U = 31$, $U'_{\alpha=0.05,n1=6,n2=6} = 29$, $p < 0.05$). Increase in absorption in particulate fraction exposed to UV-B + PAR was significantly higher from the control (PAR) at all wavelengths (Mann–Whitney *U*-test, *U* for 310 nm = 31, *U* for 330 nm = 32, *U* for 360 nm = 34, $U'_{\alpha=0.05,n1=6,n2=6} = 29$, $p < 0.05$)

Time (h)	310 nm	330 nm	360 nm
UV + PAR			
POM			
0	3.94	3.82	4.47
6	4.20	4.11	5.09
12	4.16	4.06	4.68
24	6.76	6.50	8.29
36	6.22	5.77	6.93
48	6.22	5.77	6.93
60	6.82	6.64	7.62
DOM			
0	3.38	0.99	0.84
6	4.06	1.27	0.92
12	4.83	1.61	1.22
24	3.59	0.90	0.81
36	4.27	1.53	1.13
48	4.80	2.13	1.71
60	4.06	1.45	1.04
PAR			
POM			
0	3.94	3.82	4.47
6	4.2	4.05	4.61
12	4.54	4.12	4.97
24	3.55	3.29	3.91
36	3.97	3.65	4.38
48	4.73	4.13	4.80
60	2.86	2.43	3.03
DOM			
0	3.38	0.99	0.84
6	3.31	0.76	0.47
12	3.09	0.28	0.21
24	3.32	0.67	0.22
36	4.46	1.49	0.94
48	4.61	1.68	1.07
60	4.40	1.43	0.78

and in the cells. The difference between the absorption of the initial GPM medium and the filtrate from the *L. polyedra* culture show that the 362 nm peak will develop as a result of the addition of compounds with absorption properties similar to those of the MAAs (Fig. 2: insert). Chromatographic analysis confirmed the presence of MAAs in the dissolved organic fraction (DOM), with peaks corresponding to those found in the cells (Fig 3). A positive linear correlation was found between total absorption in the *L. polyedra* filtrate between 300 and 400 nm (area under the curve) and the sum of the MAAs peak area from HPLC analysis ($r^2 = 0.83$, $F = 1917.95$, and $P < 0.015$, with $n = 3$).

The particulate absorption was always higher than the dissolved fraction after an initial lag time in Experiment 1 (Table 1 and Fig. 4). We observed an increase in net UV absorption in the particulate fraction, with a lower increase in absorption by the dissolved fraction (Fig. 4a, b, c). In control samples irradiated with only PAR (Fig. 4d, e, f), absorbance in the dissolved fraction was sometimes higher than in the particulate fraction, although no particular trend was observed. We tested the null hypothesis that absorption in the filtrate or particulate fractions would not differ between the two treatments. Absorption in the filtrate was significantly higher at 360 nm when exposed to UV-B + PAR, while particulate absorption was significantly higher at the three wavelengths measured (Table 1). Under either UV-B + PAR or PAR, chlorophyll *a* concentration showed no significant change. Average increase in chlorophyll *a* concentration was 0.92 mg chl m^{-3} after 72 h of incubation, from an initial concentration of 20.9 mg chl m^{-3}.

The exudation of UV-absorbing compounds was measured as the rate of change in absorption of the filtrate. Absorption changes in DOM (ΔAbs DOM) in cultures treated with UV-B compared to samples without UV-B (Abs quartz minus Abs Plexiglas) is inversely related to the change in intracellular concentration of these same compounds in the particulate fraction (ΔAbs POM), calculated in the same manner (Fig. 5). This relationship can be described as:

$$\Delta\text{Abs DOM} = 0.04 + 0.82 * \exp^{-(\Delta\text{Abs POM})}$$

$$r^2 = 0.69 \qquad (3)$$

DOM degradation can be expressed as (Plane et al. 1987):

$$\text{Total removal} = \text{thermal} + \text{photochemistry} + \text{biology}. \qquad (4)$$

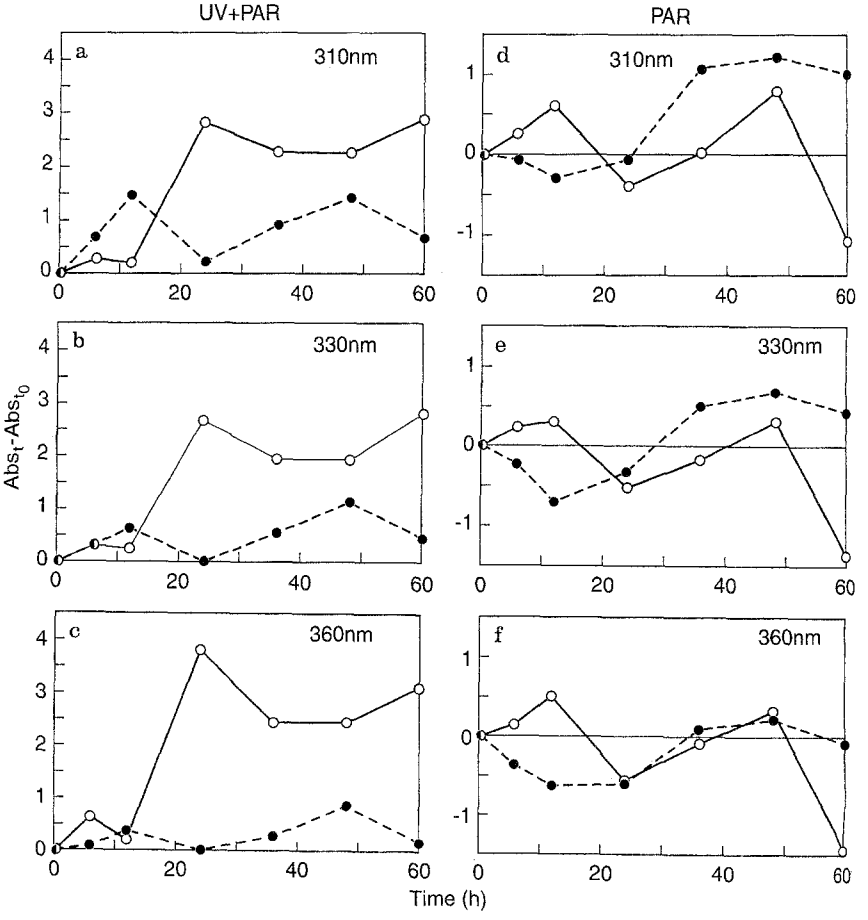

Fig. 4 *Lingulodinium polyedra*. Net change in absorption of cells (POM, ○) and filtrate (DOM, ●): Experiment 1. All graphs normalized by initial absorption. **a–c** Cultures exposed to UV-B and PAR radiation; **d–f** control cultures shielded by Plexiglas (UF-3) and thereby only exposed to PAR radiation

DOM exuded by *Lingulodinium polyedra* and exposed to UV-B and PAR radiation shows decay over a 24 h period as a result of all three loss factors in Eq. (4) (Fig. 6a). The thermal + biology components were measured by the change of absorption in a dark bottle. This loss accounted for one-third of the total decrease in absorption in a 48 h time period (Fig. 6b), suggesting that photooxidation was the largest loss term.

Time-series of DOM absorption in the filtrate exposed to UV-B + PAR are presented in Table 2.

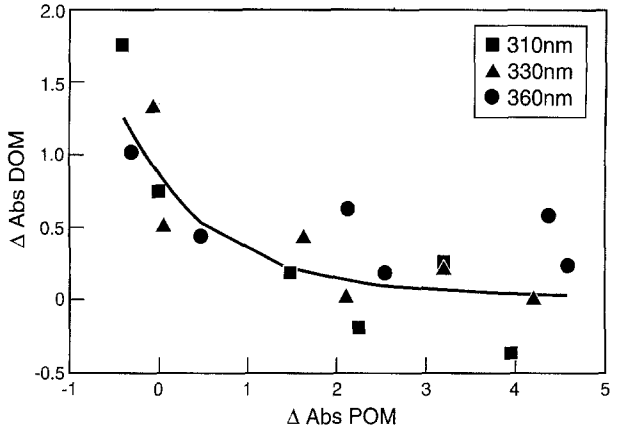

Fig. 5 *Lingulodinium polyedra.* Net release of UV-absorbing compounds in Experiment 1 as a function of intracellular concentration of same compounds, as indicated by intensity of absorption

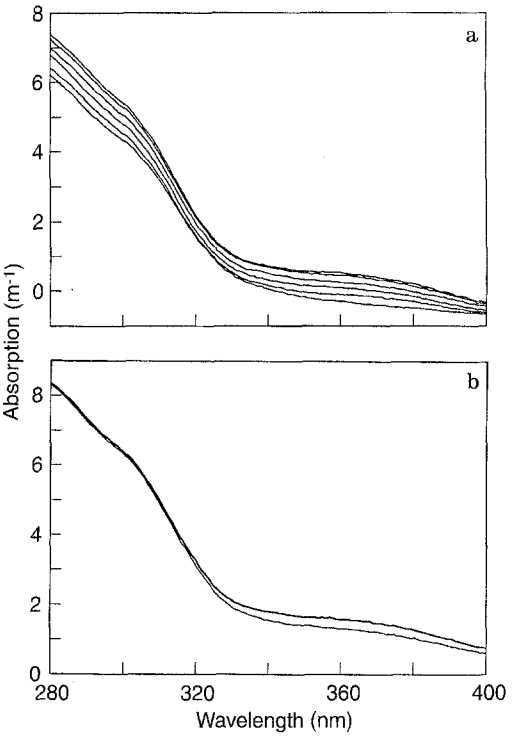

Fig. 6 *Lingulodinium polyedra.* Changes observed in absorption spectrum of filtrate exposed to UV-B and PAR radiation (Experiment 3). **a** Absorption of filtrate (DOM) over 24 h interval; from top to bottom, each line represents new time point in series from 0 to 24 h. **b** Same filtrate placed in dark displayed net degradation during 48 h interval

Table 2 *Lingulodinium polyedra.* Time-series of absorption (m^{-1}) in dissolved organic matter (DOM) as a function of time during three photooxidation experiments exposed to 0.127 W m^{-2} of UV and 30.9 µmol quanta $m^{-2} s^{-1}$ of PAR. DOM was produced by culture of *L. polyedra* during exposure to UV-B + PAR

Time (h)	UV + PAR			PAR		
	310 nm	330 nm	360 nm	310 nm	330 nm	360 nm
Experiment 2						
0	5.35	3.26	2.37	5.35	3.26	2.37
1	4.55	2.49	1.82	4.29	2.26	1.56
2	4.38	2.34	1.60	4.00	2.13	1.54
4	4.76	2.65	1.85	4.55	2.43	1.64
8	4.08	2.13	1.42	4.17	2.12	1.48
24	3.96	1.96	1.19	4.63	2.58	1.80
48	3.87	2.18	1.27	5.99	3.45	2.35
Experiment 3						
0	5.02	2.06	1.54	5.02	2.06	1.54
1	4.92	2.02	1.48	4.68	1.81	1.32
2	4.73	1.83	1.28	4.59	1.66	1.22
4	4.49	1.67	1.11	4.59	1.66	1.16
8	4.27	1.54	0.92	5.14	2.15	1.43
24	4.16	1.49	0.73	6.36	2.60	1.79
48	4.80	2.28	1.60	4.95	1.92	1.22
Experiment 4						
0	6.25	1.62	0.61	6.25	1.62	0.61
1	6.23	1.61	0.62	6.27	1.65	0.67
2	6.05	1.48	0.49	6.12	1.53	0.55
4	6.06	1.56	0.55	6.21	1.65	0.68
8	6.00	1.47	0.51	6.27	1.69	0.74

Table 3 *Lingulodinium polyedra.* Photooxidation rates of dissolved organic matter (DOM) released as a function of total dose for UV-B + PAR and PAR exposures. Filtrate obtained after cells were removed from culture medium by filtration through combusted glass-fiber (Whatman GF/F) filter. Rates were estimated for entire experiment (48 h in Experiments 2 and 3 and 8 h in Experiment 4). Photooxidation rate constant, $k(\lambda)$ (m^2 mol^{-1}), was calculated based on total dose, and time of exposure, $\kappa(\lambda)$ (s^{-1}), was based on time for filtrates exposed to irradiance of 0.527 µmol quanta $m^{-2}s^{-1}$ of UV and 30.9 µmol $m^{-2}s^{-1}$ of PAR

Wavelength (nm)	UV + PAR			PAR	
	$k(\lambda)$ (m^2 (mol quanta)$^{-1}$)	$k(\lambda)\cdot 10^6$ (s^{-1})	r^2	$k(\lambda)$ (m^2 (mol quanta)$^{-1}$)	r^2
Experiment 2					
310	− 0.14	− 1.4	0.50	− 0.13	0.22
330	− 0.23	− 1.5	0.31	− 0.20	0.32
360	− 0.26	− 2.7	0.53	− 0.22	0.36
Experiment 3					
310	− 0.04	− 1.9	0.69	− 0.02	0.69
330	− 0.08	− 3.4	0.64	− 0.05	0.71
360	− 0.11	− 8.1	0.84	− 0.05	0.28
Experiment 4					
310	− 0.006	–	0.68	− 0.005	0.04
330	− 0.013	–	0.55	− 0.012	0.83
360	− 0.040	–	0.37	− 0.020	0.49

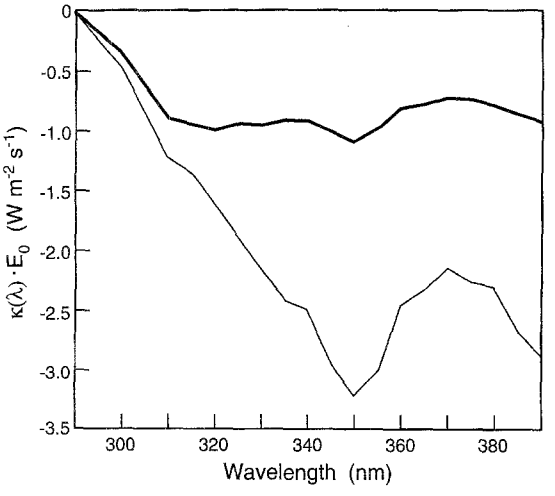

Fig. 7 Photooxidation rate constants [$\kappa(\lambda)$, s^{-1}] over 48 h of experiment, as estimated by Kouassi and Zika (1992) as a function of wavelength for Experiments 2 (*thick line*) and 3 (*thin line*)

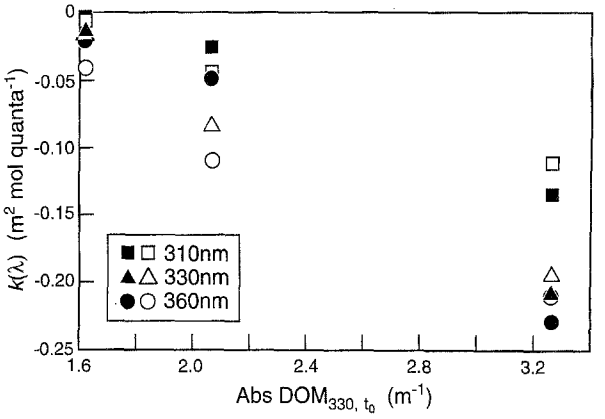

Fig. 8 *Lingulodinium polyedra.* Rate of photooxidation [$k(\lambda)$, m^2 (mol quanta)$^{-1}$] as a function of initial concentration of DOM at 330 nm in cultures. Photooxidation rates in cultures placed under Plexiglas shield (*filled symbols*) and under UV-B + PAR (*open symbols*). Photooxidation rates were significantly different at the three initial concentrations (Kruskal–Wallis, $H = 14.362$, $H'_{\alpha=0.05,n1=6,n2=6,n3=6} = 5.801$; $p < 0.05$). Multiple-comparison tests revealed three initial concentrations to be significantly different from each other (difference between Experiments 2 and 3 = 2.905, between Experiments 2 and 4 = 5.353, and between Experiments 3 and 4 = 2.447; $p < 0.05$).

Photooxidation rates [$k(\lambda)$] as a function of the total dose irradiance for UV-B + PAR and PAR treatments (Experiments 2, 3 and 4) are shown in Table 3. Photooxidation rate estimates range from 0.006 to 0.26 m^2 (mol quanta)$^{-1}$. In general, higher photooxidation rates were observed at longer wavelengths (> 330 nm). Overall, UV-B radiation was more or as effective as PAR. Photooxidation constants as a function of time [$\kappa(\lambda)$, s^{-1}] vary from $-1.4\cdot10^{-6}$ to $-8.1\cdot10^{-6}$ s^{-1} (Table 3) and are wavelength-dependent (Fig. 7), as estimated in the field by Kouassi and Zika (1992). Overall, photooxidation rates were inversely proportional to the initial DOM concentration, as measured by the absorption. This relationship was linear over the range of concentrations studied (1.6 to 3.4 m^{-1}: Fig. 8). We tested the null hypothesis that photooxidation rates were independent of the initial absorption in the filtrate. Photooxidation rates were significantly different at all three initial concentrations (Fig. 8).

Discussion

Our results show that UV-absorbing compounds synthesized by *Lingulodinium polyedra* can be released to the surrounding medium, where they remain in solution. The resemblance in absorption spectra of the particulate and dissolved matter suggested a similar composition in both carbon pools. The similarity is most evident in the shoulder in absorption observed at 360 nm (Fig. 2), attributed in part to MAAs (palythene, absorption maxima at 360 nm: Fig. 3). Confirmation of the presence of MAAs, both in the POM and the DOM in the region from 320 and to 364 nm, was obtained by chromatography (Fig. 3).

The synthesis of MAAs in the cells was highest under UV-B radiation, as observed before for this species (Carreto et al. 1990a). In addition, increases in the tyrosine- and phenylalanine-dissolved intracellular and total cellular amino acid pools exposed to UV radiation have been observed (Goes et al. 1995). Aromatic amino acids, such as tyrosine and phenylalanine, are synthesized in the Shikimate pathway and are precursors to MAA synthesis. Thus, an increase in MAA concentration under UV photoinhibition is expected. Absorption by DOM in the filtrate increased slightly under UV-B compared to PAR. These results are to be expected, as DOM production by phytoplankton is a well-known process (Zlotnik and Dubinsky 1989; Lancelot 1983), and MAAs can only be part of the overall excretion.

The release of MAAs by *Lingulodinium polyedra* was not a simple function of intracellular concentration of the same compounds. Rather, an inverse relationship between the POM and DOM pools was observed, whereby extracellular release depleted the cells of MAAs, resulting in a lowering of absorption in the cellular fraction (Fig. 5). At other times, the MAAs accumulated intracellularly and the exudation decreased. Given that MAAs in the surrounding medium accumulated faster during the first 24 h, before the MAAs accumulated intracellularly, it can be argued that healthy cells adapted to UV-B synthesize MAAs, while cells in shock and/or recently exposed to UV-B will not grow actively and will release MAAs. Although our cell counts and chlorophyll *a* measurements did not show net death, we cannot rule out the possibility that the release of MAAs was a consequence of cell lysis. This hypothesis will have to be tested using methods to detect viable vs dead cells. Independent of the mechanism by which MAAs become part of the DOM, the results presented here suggest that a fraction of the MAAs synthesized by cells are eventually present in the surrounding medium.

Photooxidation was the largest factor contributing to total degradation of DOM, with the thermal and biology components accounting for only one-third of the total (as estimated in a dark bottle: Fig. 6). It has been suggested that biologically usable carbon produced from DOM degradation may enhance bacterial growth (Mopper and Zhou 1990), causing irradiated samples to have a slightly higher biological component than that reported for the dark bottle. Even with the underestimation that increased biological activity would introduce, photooxidation would probably remain the greatest loss factor. The experimentally determined photooxidation rates, $\kappa(\lambda)$, calculated in this study are three times higher than those found by Kouassi and Zika (1992) in the field (Table 3). This difference may be a consequence of the present experimental conditions in which the UV-B:PAR ratio was three times higher than that found in natural sunlight.

The linear relationship between the initial DOM concentration and the photooxidation rate $[k(\lambda)]$ observed, accounts for the high amount of variability in photooxidation rates between experiments (Fig. 8) and suggests hydrolysis (Mopper and Zhou 1990; D. Wolgast, Scripps Institution of Oceanography, personal comm.). Variations in DOM composition and source and the history of the water affect degradation rates and could also add to experimental variations (Dister and Zafiriou 1993). In addition, decay of the MAAs in seawater was wavelength-dependent, with higher rates being observed at longer wavelengths (Table 3). This dependence may be due to the decay of compounds, with absorption at longer wavelengths resulting in an increase in absorption at shorter wavelengths (Kouassi and Zika 1990).

Overall, UV radiation in the water column is a function of incident irradiance, and its extinction by particulate and dissolved matter, and the water itself. Absorption by particulate matter is considered to be the main source of variability in the visible while DOM absorption dominates in the UV (Bricaud et al. 1981). Measurements of DOM absorption in open waters show an exponential decrease towards longer wavelengths. Absorption properties of fresh DOM, as measured in this study, reveal a more complex structure, in particular the presence of chromophores at 362 nm. Presumably these chromophores are not observed in open waters, and their presence is no longer detectable because they have been degraded and/or are too dilute to detect, although they have been measured under conditions of high phytoplankton concentration i.e., dinoflagellate blooms (Whitehead and Vernet 1996).

In summary, the observed release of UV-absorbing compounds with high UV-A absorption properties, and the photooxidation rates measured for newly released material, suggest that phytoplankton such as *Lingulodinium polyedra* may be an important source of DOM in marine waters and that we might expect buildup of DOM after high dinoflagellate concentrations such as red tides (Whitehead and Vernet 1996). Under our experimental conditions, with UV-B radiation three times higher than the expected irradiance in a summer day at mid-latitudes, daily consumption

rates due to photooxidation were on the order of 3 to 10% of the DOM produced. At these rates, MAAs in the DOM may contribute to the decrease of UV transmission in the water column on a time scale representative of phytoplankton growth (days) and the development of blooms (weeks).

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