

Influence of mycosporine-like amino acids (MAAs) on UV absorption by particulate and dissolved organic matter in La Jolla Bay

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Abstract

Experimental work with cultures of the red tide dinoflagellate *Lingulodinium polyedrum* suggested mycosporine-like amino acids (MAAs) are a component of the organic matter excreted by cells. MAAs in dissolved organic matter (DOM) also may have a large influence on absorption of ultraviolet (UV) light through the water column. To test these hypotheses in a natural setting, data were collected from March 1995 through April 1996 in coastal waters off California, U.S.A. During this time, a large red tide of *L. polyedrum* occurred in March and April 1995. In this field study, we show that MAAs are a quantifiable component of DOM, particularly during the period following the red tide events. Absorption spectra of particulate organic matter (POM) and DOM samples revealed disproportionately high levels of UV absorption relative to visible wavelengths. UV absorption by POM at 330 nm was linearly related to phytoplankton biomass (between 0–10 μg chlorophyll [Chl] $a\text{ L}^{-1}$), measured as Chl *a*, accounting for 71% of the variability in absorption. Chromatographic analyses revealed the presence of various MAAs in both POM and DOM pools. MAAs were observed in 83% ($n = 53$) and 47% ($n = 13$) of the samples analyzed with concentrations ranging from 0 to 2.75 μM (0–696.00 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*) in the POM and 0 to 111.40 nM in the DOM fraction. Absorption by dissolved MAAs, as calculated from the measured concentrations, correlated with DOM UV absorption ($r^2 = 0.77$) and accounted for up to 10% of the total DOM absorption at 330 nm. Thus, MAAs are a small but quantifiable component of the DOM pool in the field and contribute to UV absorption.

Unusually high ultraviolet (UV) attenuation observed during red tides of *Lingulodinium polyedrum* (formerly known as *Gonyaulax polyedra*; Dodge 1989) has been attributed to the presence of mycosporine-like amino acids (MAAs; Vernet et al. 1989; Kahru and Mitchell 1998). These compounds have been identified in a wide phyletic assortment of marine organisms and may provide partial photoprotection from ultraviolet (UV) radiation (Karentz et al. 1991; Bandaranayake 1998; Jeffrey et al. 1999). *L. polyedrum* produces seven types of MAAs with absorption maxima ranging between 310 and 364 nm (Vernet and Whitehead 1996). It has been hypothesized that in a natural setting, MAAs are released by dinoflagellates and become incorporated into the dissolved organic matter (DOM) pool (Vernet and Whitehead 1996).

Phytoplankton release a variety of organic compounds in response to many environmental factors, including nutrient limitation and changes in light quantity or quality (Berman and Holm-Hansen 1974; Verity 1981; Lancelot 1983; Zlotnik and Dubinsky 1989; Braven et al. 1995). Phytoplankton are capable of releasing 10–60% of the carbon and 15–50%

of the nitrogen assimilated during photosynthesis (Sundh 1992; Bronk et al. 1994; Braven et al. 1995; Malinsky-Rushansky and Legrand 1996; Slawyk et al. 1998). Thus, DOM concentrations can significantly increase after phytoplankton blooms, such as those composed of diatoms or *Phaeocystis pouchetii* (Ittekkot 1982; Billen and Fontigny 1987). Although red tides increase the total amount of DOM (Holmes et al. 1967), changes within chemical composition of DOM may be harder to detect. The potential for shifts in the distribution of minor chemical components of the DOM pool (e.g., MAAs) to affect a bulk property such as absorption is an intriguing issue.

In most aquatic environments, DOM is largely responsible for regulating the penetration of UV radiation through the water column (Smith and Baker 1979). In coastal areas, the concentration of DOM is mainly regulated by terrestrial input, zooplankton feeding, and phytoplankton production (Lee and Wakeham 1988; Lee and Henrichs 1993; Mann and Wetzel 1995; Hedges et al. 1997). Since an area such as La Jolla Bay, California has no major riverine source and only receives runoff from episodic rain events, it may be possible to detect changes in DOM absorption that is due to phytoplankton material input. During a red tide in La Jolla Bay in the mid-1960s, dissolved organic carbon concentrations increased by ~70% as a result of phytoplankton production (Holmes et al. 1967). Accumulation of DOM originating from exudates released by phytoplankton may increase UV attenuation by the dissolved pool, thus providing a UV shield during these shallow blooms. The presence of MAAs in the exudates has the potential to further increase the attenuation of UV radiation (310–380 nm), offering even greater protection.

During March and April 1995, an unusually strong *L. polyedrum* red tide occurred off the southern California coast,

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extending from San Diego northward ~200 km. Red tide events have been observed in this area since 1901 and recur every few years (Torrey 1902; Allen 1938, 1943; Holmes et al. 1967). During this 1995 red tide, chlorophyll (Chl) *a* concentrations in excess of 100 $\mu\text{g Chl } a \text{ L}^{-1}$ were recorded in surface waters. Because the bulk of the biomass was found in the upper few meters of the water column, cells were exposed to high levels of visible and UV radiation. Bio-optical profiles taken during the red tide showed unusually low UV reflectance at 340 and 380 nm, which was attributed to the presence of MAAs produced by *L. polyedra* (Kahru and Mitchell 1998).

Here we present data from a year-long study beginning during this massive red tide event on 31 March 1995. Sampling from the pier at Scripps Institution of Oceanography continued on a weekly basis through 4 April 1996. Chromatographic analysis of both particulate and dissolved fractions was conducted to determine whether MAAs were present in the particulate organic matter (POM) and DOM pools and whether MAAs could account for variations in surface water UV attenuation. We also examined the relationship between UV absorption in the DOM and POM pools and Chl *a* concentration.

Methods

All samples were taken from Scripps pier beginning on 31 March 1995, continuing through 4 April 1996 on a weekly basis. Sampling was conducted at the same time of the morning from the end of the pier with a Wally bottle after a 3-min equilibration period from approximately 1 m deep. The water sample was then transferred to a prerinsed, dark 1-liter Nalgene bottle.

Samples were prepared for analysis within 6 h of being brought from the pier. Replicate samples ($n = 2$) were concentrated by differential pressure (<5 psi) onto combusted Whatman GF/F filters (4 h at 500°C); the filtrate from the replicate samples was combined and collected into amber Quorpak bottles (one filtrate sample per sampling day). The volume filtered varied depending on the amount of biomass in the whole water sample but was generally 60–900 ml for the POM samples, with 120 ml of the filtrate kept in combusted bottles for the DOM sample. Filtrate (DOM samples; $n = 53$) and filters (POM samples; $n = 53$, for a total of $n = 106$ with replicates) were kept at -80°C until analysis. Spectrophotometric measurement of all samples was conducted within 1 month of sampling. Absorption properties of POM and DOM were measured as in Vernet and Whitehead (1996). The coefficient of variation between replicate POM absorption samples was 18.4% at 310 nm, 20.7% at 330 nm, and 21.3% at 360 nm because of patchiness on the GF/F filter pads. From January through March 1996 (eight samples total), absorption of detrital matter (a_d) was also measured by removal of phytoplankton pigments (and MAAs) via extraction in warm methanol (Kishino et al. 1985). Using the measured detrital value, absorption by phytoplankton (a_{ph}) was calculated as the difference between total particulate (POM) absorption and detrital absorption. Absorption spectra were baseline corrected using 740–750 nm as the null point.

Prior to MAA analysis via high-performance liquid chromatography (HPLC), 1–3 ml of HPLC-grade methanol was added to the particulate sample, and the filter was ground on ice. The mixture was allowed to extract overnight at 4°C and was clarified by filtration through a prerinsed 0.2- μm sterile filter prior to analysis. Extraction efficiency for POM samples was 99%, as determined by successive extractions until MAAs were no longer detectable in the extract. A total of 48 POM samples from different sampling dates were analyzed for MAAs plus replicates of five samples for a total of 53 analyses. Analysis of the replicate samples yielded a variation of less than 3%. Repeat injection and analysis from the same extract varied by less than 1%.

The filtrates (DOM) from the POM samples were prepared for MAA analysis by concentration and extraction on a C-18 cartridge (Maxi-Clean SPE Cartridge, 300 mg of C-18 packing; Alltech Associates) and eluted with 100% methanol (Vernet and Whitehead 1996). The C-18 cartridge was first activated by slowly flushing with 5 ml of water followed by 5 ml of methanol. The dissolved sample (60 ml) was then concentrated onto the cartridge and eluted by three subsequent 1-ml volumes of methanol. The MAAs were present in the second aliquot, as determined by spectrophotometric analysis of all three fractions. This extraction and subsequent HPLC analysis were performed approximately once a month on 17 of the 53 DOM samples. Four of the 17 DOM samples were analyzed prior to our ability to quantify MAAs, resulting in 13 dissolved MAA samples for which concentrations could be calculated.

The extraction efficiency was calculated by following the extraction procedure outlined above using a solution of shinorine and porphyra-334 standards at a concentration of 350 nM. Extraction efficiency was calculated as the difference between MAA concentration in the initial solution before passage through the C-18 cartridge and in the effluent after passage through the cartridge. The MAA concentration in the methanolic eluent was also measured and agreed with the concentration obtained by difference. The average measured extraction efficiency was $7.4 \pm 0.2\%$, and a general correction factor of 90% was applied in the calculation of dissolved MAA concentrations. The resulting concentrations for dissolved MAAs are consistent with the expected concentration of MAAs required to generate the shoulder in UV absorption often seen in DOM absorption spectra. Hence, the data are internally consistent.

Chromatographic conditions were similar to those described elsewhere (Dunlap et al. 1986; Vernet and Whitehead 1996). During HPLC analysis, the methanolic extracts from particulate and dissolved samples were eluted with 75:25:0.1 methanol:water:acetic acid off a Brownlee Spheri-5 RP-8 column at a flow rate of 0.8 ml min^{-1} . The HPLC column was re-equilibrated between samples with 80:20 methanol:water for 10 min. The separation was carried out in a Perkin Elmer system with a Perkin-Elmer LC-235 Diode array detector (scanning from 280–400 nm), recording absorption at 315 and 340 nm. The analytical detection limit for both dissolved and particulate extracts was 0.01 nM.

Individual peaks were identified by retention time, absorption properties, and cochromatography. Secondary standards used for aid in peak identification (but not quantifi-

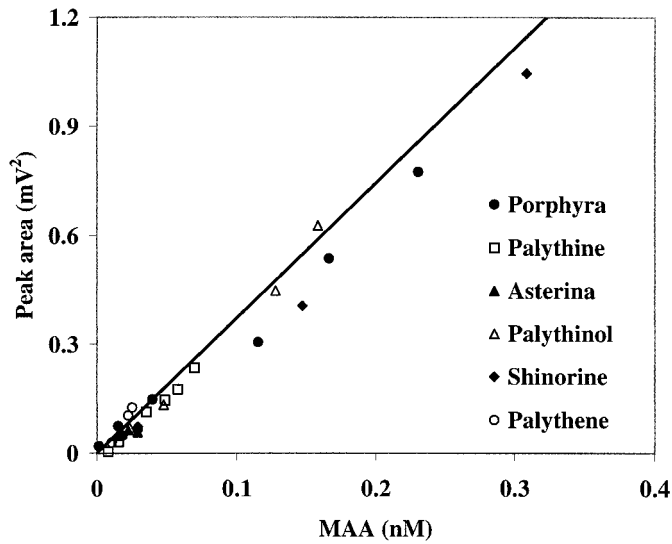


Fig. 1. Standard curve for calculation of mycosporine-like amino acid (MAA) concentration from peak area (detector set at 315 nm). Individual MAA standards are indicated by different symbols. The solid line indicates the linear fit to the combined standards ($r^2 = 0.99$; $n = 25$).

cation) were from *Curdise racovitae*, *Palmaria decipiens*, and a subtidal Anthozoa (anemone #1 as in Karentz et al. 1991): mycosporine-glycine, $R_t = 3.51$ min, $Abs_{max} = 309$ nm; shinorine, $R_t = 5.43 \pm 0.08$ min, $Abs_{max} = 334$ nm; porphyra-334, $R_t = 6.42 \pm 0.23$ min, $Abs_{max} = 334$ nm; mycosporine-glycine-valine, $R_t = 7.43 \pm 0.12$ min, $Abs_{max} = 336$ nm; asterina-330, $R_t = 7.95$ min, $Abs_{max} = 330$ nm; palythine, $R_t = 8.08 \pm 0.31$ min, $Abs_{max} = 322$ nm; palythiol, $R_t = 12.76$ min, $Abs_{max} = 332$ nm; and palythene, $R_t = 13.44$ min, $Abs_{max} = 362$ nm.

Quantitative calibration of the HPLC system was achieved using authenticated standards from Hideshi Nakamura (Nogoya University, Japan; porphyra-334, shinorine, palythine, asterina-330, and palythiol). Concentrations of the standards in water were calculated spectrophotometrically using published molar extinction coefficients (Takano et al. 1978a,b; Banaszak and Trench 1995). A known volume of standard was removed, evaporated to dryness under nitrogen gas, redissolved in 100% methanol, and analyzed chromatographically to obtain peak areas using the method described above at the 315-nm detector setting. The calibration line was generated by dilution and HPLC analysis of the methanol standard. Peak areas were calculated using EZ Chrome software. Since only 5 MAA standards were available and the field data contained many unknown compounds with chromatographic properties and wavelength maxima similar to MAAs ($310 \text{ nm} < \lambda_{max} < 360 \text{ nm}$), the calibration lines from the 5 MAA standards were combined to give a single calibration (Fig. 1; $n = 25$; $r^2 = 0.99$). We applied this calibration to both the known MAAs and the unknown UV-absorbing compounds. Combining the 5 MAA standards into one calibration equation did not significantly change the calculated concentration for the MAA standards. This calibration, however, assumes that the unidentified peaks are MAA-like compounds (or MAAs for which we do not have

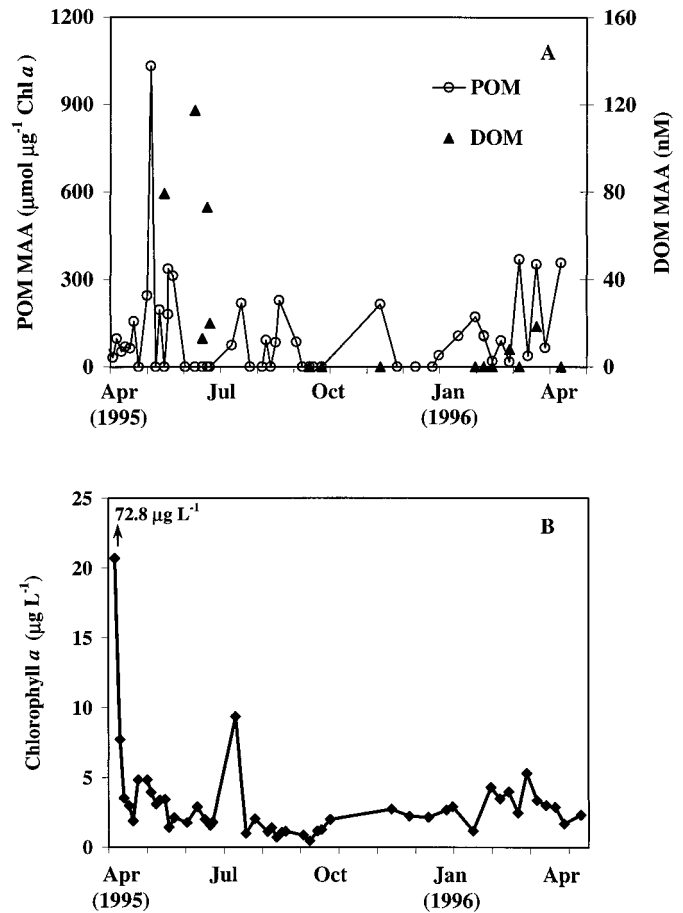


Fig. 2. (A) Time series of total MAA concentration of POM (open circles, $\mu\text{mol } \mu\text{g}^{-1} \text{ Chl } a$) and DOM (filled triangles, nM) samples. (B) Biomass, as estimated by Chl *a* (filled diamonds, $\mu\text{g Chl } a \text{ L}^{-1}$).

standards) with extinction coefficients similar to those of the characterized MAAs.

Chl *a* concentrations were obtained from J. McGowan and C. L. Fey of Scripps Institution of Oceanography. Sampling occurred simultaneously with the collection of POM and DOM samples. The analytical procedure followed Holm-Hansen et al. (1965). Samples were collected in the same bottle cast as the samples described above and were concentrated onto GF/F filters and extracted in 90% acetone. Extracts were kept for 24 h in the dark at 3°C before analysis on a Turner Designs fluorometer calibrated with a Chl *a* standard (Sigma Co.).

Results

MAAs were most abundant during the spring bloom in the particulate fraction ranging from 0.48 to 2.75 μM (6.6–149.8 $\mu\text{mol } \mu\text{g}^{-1} \text{ Chl } a$), and postbloom in the dissolved fraction ranging from 3.43 to 111.40 nM (Fig. 2). In the months following the bloom, MAAs were measured at decreasing concentrations in the POM (0.0–91.7 $\mu\text{mol } \mu\text{g}^{-1} \text{ Chl } a$) but were detectable in 83% of the weekly samples taken over the year-long sampling period ($n = 53$). In the

Table 1. MAAs and unidentified UV-absorbing compounds (lettered A through N) from HPLC analysis of POM and DOM samples are listed below. (Numbers in parentheses indicate how many times the compound was detected over the year-long study.) Wavelength maxima and retention times are given with the standard deviation. NQ, not quantifiable.

MAA	λ_{\max} (nm)	Retention time (min)	POM	DOM
			($\mu\text{mol MAA } \mu\text{g}^{-1}$ Chl <i>a</i>)	(nM)
Mycosporine–glycine	310±2	3.56±0.17	37.90–218.50 (17)	18.51 (1)
Shinorine	334±1	5.38±0.26	53.10–73.10 (4)	—
Porphyra	334±1	6.51±0.23	6.64–59.49 (7)	3.43–26.03 (3)
Mycosporine–glycine–valine	335±1	7.60±0.30	10.76–100.42 (15)	—
Palythine	321±2	7.93	—	111.40 (1)
Palythene	362±2	13.18±0.47	15.51–24.44 (4)	—
A	333±1	4.77±0.07	24.01–696.00 (2)	—
B	324±1	4.82±0.11	3.73–336.13 (15)	8.87–79.22 (2)
C	326±1	5.14±0.35	3.53–85.37 (4)	—
D	328±1	5.25±0.25	26.05–49.44 (7)	9.58–46.90 (3)
E	335±1	9.76±0.47	6.66–20.79 (3)	8.01 (1)
F	324	10.63±0.19	NQ (2)	102.10 (1)
G*	320	10.84±0.44	20.85 (2)	18.73 (1)
H	364	10.85±0.03	2.43–5.86 (2)	—
I*	325	11.14±0	21.93 (2)	—
J	335	11.92±0.01	33.78–88.14 (3)	—
K	365±1	11.98±0.25	3.04–11.22 (2)	—
L	320	14.41±0.32	17.25–132.31 (4)	—
M	325±1	14.33±0.40	11.80–75.74 (4)	—
N	322±1	15.92±0.06	107.89–171.23 (2)	—

* Only one quantifiable sample.

dissolved fraction, MAAs were also detectable during the summer at lower concentrations (8.87–10.01 nM) and were present in 47% of the DOM samples analyzed ($n = 13$).

The composition of MAAs in POM and DOM correlated such that at least one specific MAA (or unknown UV-absorbing compound) was common to both fractions from the same sampling day with two to three UV-absorbing compounds typically present. During the red tide (March–April 1995), several MAAs were found in the particulate fraction, including mycosporine–glycine, shinorine, and porphyra-334, with concentrations ranging from 6.6 to 149.8 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*. These same MAAs were present throughout the year-long sampling period at lower concentrations. Mycosporine–glycine (37.9–101.7 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*) and mycosporine–glycine–valine (10.7–100.4 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*) were the dominant MAAs from September 1995 through the end of the study period in April 1996, with palythene (15.5–24.4 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*) detectable in four instances. Generally, MAA concentrations were lower during nonbloom periods, with the exception of 1 week in July, where mycosporine–glycine and mycosporine–glycine–valine were measured at 218.5 and 41.0 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*, respectively. During the red tide bloom, several unidentified compounds (labeled A, B, C, E, H, K; Table 1) were also detected in the POM, with combined concentrations ranging from 2.4 to 696.0 $\mu\text{mol } \mu\text{g}^{-1}$ Chl *a*. Compound B ($\lambda_{\max} = 324$ nm, $R_t = 4.82$ min) was the dominant unknown both during and just after the red tide bloom. A total of 14 unknown UV-absorbing compounds (labeled A–N; Table 1) were detected over the course of this study.

Highest dissolved MAA concentrations (up to 111.40 nM) were observed in May and June, with three different UV-absorbing compounds dominating: porphyra-334, palythine, and one unknown (labeled F, $R_t = 10.17$ min, $\lambda_{\max} = 324$ nm). The composition during the bloom consisted of two compounds (porphyra-334 and unknown G) at reduced concentrations (~14.3 nM). Porphyra-334 was also abundant postbloom in the dissolved phase. In total, 14 unknown compounds were identified (labeled A–N in order of retention time; Table 1) in the POM and DOM samples collected, with retention times and wavelength maxima similar to that of MAAs. Occurrence and diversity of the unknown UV-absorbing compounds was greater in the POM than the DOM. During nonbloom conditions, the concentration of unknown compounds often exceeded those measured for MAAs in both fractions.

In general, highest total spectrophotometric absorption values were measured in the spring and early summer during and just after periods of active phytoplankton growth. POM absorption dominated during the spring, with DOM absorption highest in the early summer (Fig. 3). A shoulder in UV absorption at 360 nm was observed on 13 April 1995 in the particulate fraction and in both the dissolved and particulate fractions throughout most of February 1996 (Fig. 3A,D). As illustrated in Fig. 3A (shaded area), the visible shoulder in DOM UV absorption corresponds to the absorption spectrum of MAAs, thereby suggesting the presence of these compounds at these specific sampling times (Vernet and Whitehead 1996). At other times, the presence of MAAs was not an obvious feature of the DOM absorption, as was the case

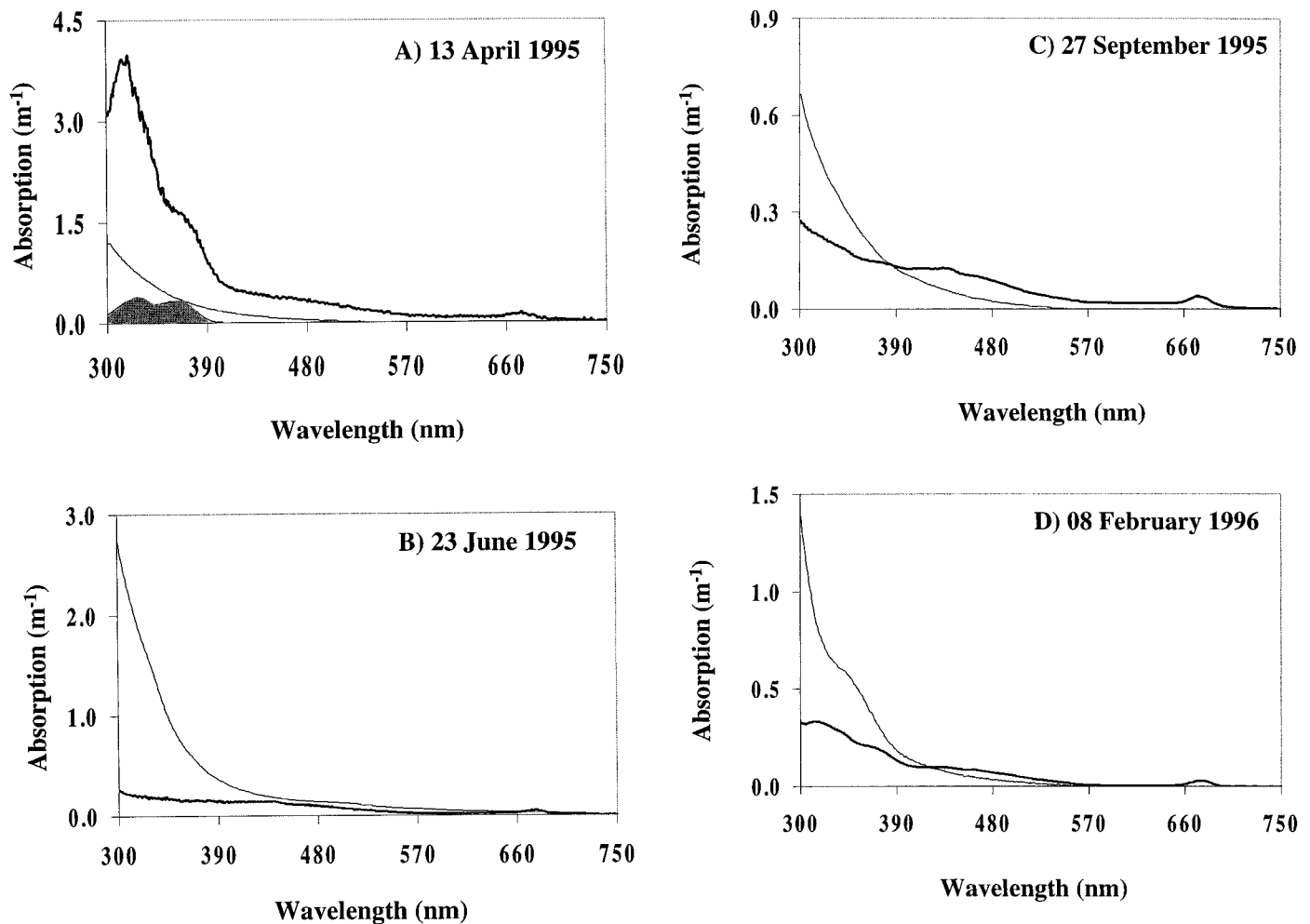


Fig. 3. Seasonal variability in absorption for particulate (thick line) and dissolved (thin line) organic matter. An example absorption spectrum of four of the MAAs identified in *L. polyedrum* (shinorine, porphyra-334, palythine, and palythene; total concentration of 3.6 μM) is shown for comparison (A, shaded area). Vertical scales vary to show features of the absorption spectra.

during June 1995 when highest MAA concentrations were measured (Fig. 2, 3B). A lag time between the peak in POM and DOM UV absorption was also observed for the red tide of 1995, as indicated by the doubling of DOM absorption between April and June 1995 after the peak in POM absorption during March (Fig. 3A,B).

Variations in UV absorption by POM were compared to phytoplankton biomass, as estimated by Chl *a* concentration. A wavelength of 330 nm was chosen as an index of UV absorption by MAAs because an increase in absorption at this wavelength has been observed in laboratory cultures of *Lingulodinium polyedrum* and because many of the compounds detected herein had wavelength maxima near 330 nm (Vernet and Whitehead 1996). Chl *a* concentrations varied from a minimum of 0.45 to a high of 72.86 $\mu\text{g Chl } a \text{ L}^{-1}$ on 6 April 1995 during the red tide. Increased particulate UV absorption at 330 nm was linearly correlated to increases in Chl *a* concentration (between 0 and 10 $\mu\text{g L}^{-1}$), accounting for 71% of the variability (thick line; Fig. 4). At high Chl *a* concentrations ($>10 \mu\text{g L}^{-1}$), the overall relationship

becomes nonlinear (thin curve, $n = 53$, $r^2 = 0.94$; Fig. 4). This relationship is primarily driven by the four high Chl *a* data points, necessitating further investigations at high biomass levels to better define this correlation.

Several laboratory studies have used key wavelengths such as 330 nm as proxies for relative MAA concentration in phytoplankton (Marchant et al. 1991; Vernet and Whitehead 1996; Wängberg et al. 1997). When comparing POM absorption at 330 nm to the concentration of MAAs in the POM, we found no significant relationship, suggesting this proxy may be confounded by other factors in field samples (Fig. 4 inset; $r^2 = 0.24$; $n = 53$). POM samples from February through April 1996 were further analyzed to remove the detrital absorption (a_d) signal. The resulting phytoplankton absorption would include only materials that were extractable in methanol, such as MAAs, photosynthetic pigments, and some amino acids. Interestingly, we still found no relationship between MAA concentration and phytoplankton absorption (a_{ph}) at 310, 330, or 360 nm ($r^2 \leq 0.15$; $n = 8$; data not shown).

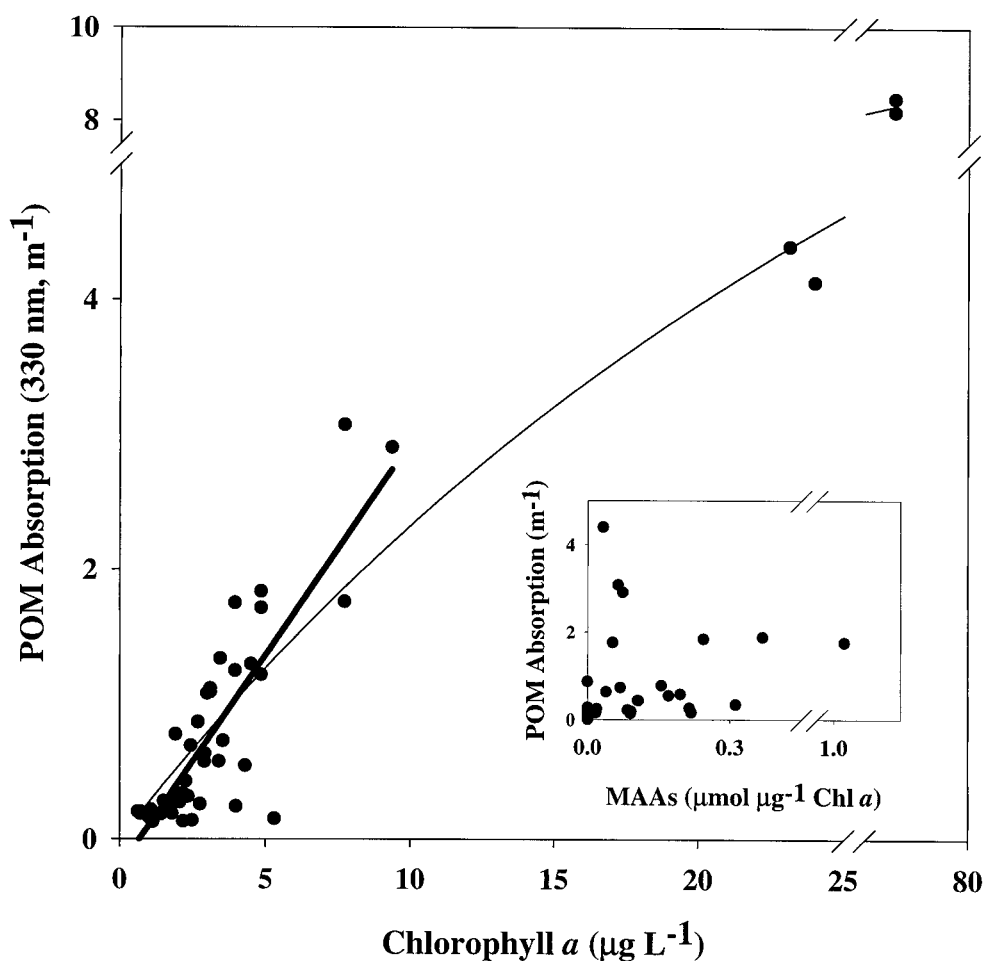


Fig. 4. Relationship between phytoplankton biomass, as estimated from Chl *a* concentrations from 0 to 10 $\mu\text{g Chl } a \text{ L}^{-1}$ and POM absorption at 330 nm (thick line, $r^2 = 0.71$; $P < 0.01$; $n = 49$). The relationship is also given between POM absorption and the entire Chl *a* range observed during the red tide bloom to 72 $\mu\text{g Chl } a \text{ L}^{-1}$ (thin curve, $r^2 = 0.94$; $P < 0.01$; $n = 53$). Total MAA concentration in the POM, however, was not related to absorption ($r^2 = 0.24$; $n = 53$; inset).

The potential for dissolved MAAs to affect DOM absorption has not previously been evaluated. The measured concentrations of MAAs and unknown MAA-like substances were converted to absorption using an average molar extinction coefficient of our MAA standards ($\epsilon = 44,000$; Takano et al. 1978*a,b*; Banaszak and Trench 1995; $n = 11$, two lost absorption data points). Fluctuations in the absorption of MAAs and unknown UV-absorbing compounds correlated with UV attenuation by the bulk dissolved pool resulting in a significant positive correlation (Fig. 5; $r^2 = 0.77$; $P < 0.01$). The equation for this relationship is

$$\text{DOM absorption} = 0.145 \cdot \text{MAA absorption} - 0.084, \quad (1)$$

where DOM absorption (m^{-1}) is taken at 330 nm and MAA absorption (m^{-1}) is the amount of absorption due solely to MAAs at the same wavelength. The absorption in DOM remaining in the absence of MAAs (MAA absorption = 0; Fig. 5) is likely a background level of UV absorption due to marine and terrestrially derived DOM. Although a minor component of the DOM on a quantitative basis (nM versus

μM concentrations), MAAs contributed up to 10% to the total absorption by DOM at 330 nm.

Discussion

Concentration and composition of MAAs—During the 1995 red tide, MAAs were thought responsible for low UV reflectance values at 340 and 380 nm in surface waters (Kahru and Mitchell 1998). HPLC analysis has confirmed the presence of MAAs in both particulate and dissolved organic matter fractions during the red tide and year-round (Table 1; Fig. 2). The observed concentration and composition of MAAs in the DOM should reflect a balance between production and removal rates. A major factor controlling MAA production is the species composition of the phytoplankton assemblage. In this area, dinoflagellates such as *Lingulodinium polyedrum* appear to be the main producers of MAAs and are most abundant during the spring (Reid et al. 1985). Intensified UV irradiance during summer months would promote an increase in MAA production, but low phytoplankton

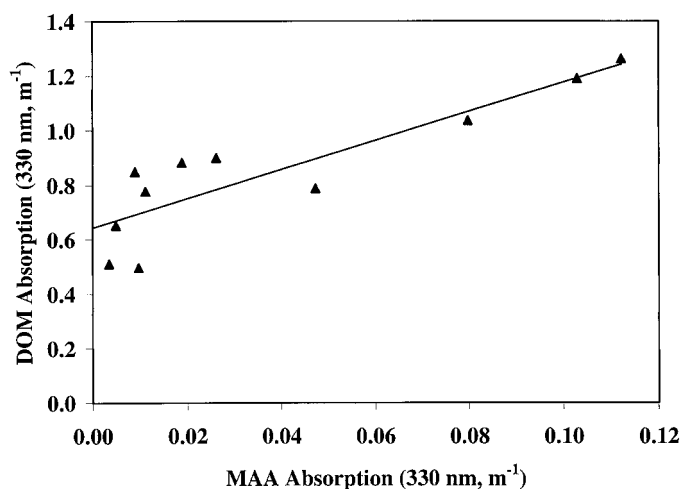


Fig. 5. Absorption at 330 nm because of dissolved MAAs (as calculated from measured MAA concentrations) is plotted against total DOM absorption at the same wavelength ($r^2 = 0.77$; $P < 0.01$; $n = 11$).

biomass and increased removal of MAAs via photodegradation or bacterial degradation could result in low or undetectable dissolved MAA concentrations (Reid et al. 1985; Bernillon et al. 1990; Vernet and Whitehead 1996; Dunlap and Shick 1998). Winter conditions of minimal phytoplankton biomass and reduced ambient UV irradiance are not conducive to MAA production and would also be expected to result in low MAA concentrations in both POM and DOM fractions.

Seven MAAs were identified using the available standards, along with 14 unidentifiable peaks (Table 1). Six MAAs and one unknown compound were previously detected in the analysis of *L. polyedrum* cultures isolated from this location (Vernet and Whitehead 1996). During the red tide bloom, three of these six MAAs were identified along with six unknown compounds (labeled A, B, C, E, H, K; Table 1). In the DOM fraction, only porphyra-334, the most abundant MAA synthesized by *L. polyedrum*, was identifiable, whereas analysis of DOM from laboratory cultures revealed all six MAAs biosynthesized by *L. polyedrum*. This difference in MAA composition between field and culture samples may affect the influence of MAAs on UV absorption in both the POM and DOM pools.

Although the potential exists for the unknown compounds present in the POM and DOM samples to be other methanol-soluble and chromatographically compatible metabolites, MAAs are currently the major biochemical class of UV-A (320–400 nm) absorbing compounds known in the marine environment (Bandaranayake 1998). The possibility also exists that these unknown compounds may be hereto unidentified MAAs, as several new MAAs are identified each year (Jeffrey et al. 1999; Karsten and Wiencke 1999; Sommaruga et al. 1999; Xiong et al. 1999), but it is unlikely that all 14 unknowns are novel MAAs, especially the unknowns detected in the DOM samples. Several mechanisms may be responsible for the conversion of known MAAs to unknown UV-absorbing compounds. Dissolved MAAs are expected to have a high chemical reactivity, undergoing various dehy-

dration reactions possibly resulting in a product with similar chemical and optical properties to the parent MAA (Takano et al. 1978a,b; Bandaranayake 1998). The structurally altered MAAs may also be the result of photochemical or bacterial degradation of these compounds, issues which merit further investigation since little is known about the biogeochemistry of these compounds (Bernillon et al. 1990; Dunlap and Shick 1998).

Influence of POM and DOM on UV absorption—The production of UV-absorbing compounds was indicated by increased absorption at 330 nm and a shoulder at 360 nm in the absorption spectra in both particulate and dissolved fractions at various times during the year-long sampling (Fig. 3A,D). *L. polyedrum* blooms in 1964 and 1965 were likewise marked by increases in dissolved organic carbon as the bloom declined rather than at the onset or peak of the bloom (Holmes et al. 1967). An inverse relationship between POM and DOM absorption was also observed in a culture of *L. polyedrum* under UV stress and was interpreted as the extracellular release of organic material, which depleted the POM fraction while increasing the absorption of DOM. Conversely, in the absence of stress while cells are actively growing, POM absorption was shown to increase while DOM absorption decreased (Vernet and Whitehead 1996). A similar mechanism may be acting in the field. Increases in POM UV absorption during March and April 1995 may be due to the additional algal biomass and also elevated amounts of detrital matter (Fig. 3A, 4). The grazing and decomposition of phytoplankton biomass later results in increased DOM absorption during June (Fig. 3B). Postbloom increases in DOM levels may decrease UV transmission through the water column, providing partial UV protection to shallow blooming phytoplankton and possibly assisting the development of a subsequent bloom following the initial source bloom.

Influence of MAAs on UV absorption—Attenuation of UV by DOM is important in minimizing the deleterious effects of UV radiation on phytoplankton (Smith and Baker 1979; Wetzel 1992; Morris et al. 1995). The composition of DOM appears to be fairly uniform throughout most of the ocean, but in coastal areas, the dissolved pool becomes much more heterogeneous because of terrestrial and riverine inputs. Increased biomass of primary producers also plays a major role in shaping the DOM pool in coastal areas (Ittekkot 1982; Billen and Fontigny 1987; Bronk et al. 1998). In our location, bulk DOM absorption at 330 nm was linearly related to dissolved MAA absorption (Fig. 5). The clearly visible shoulder in absorption at 360 nm due to MAAs illustrates the effect these compounds and other similar UV-absorbing compounds may have on UV absorption by bulk DOM (Fig. 3A, shaded area; Vernet and Whitehead 1996). Hence, despite low concentrations relative to the bulk DOM pool (nM compared to μ M), MAAs contributed up to 10% to the UV absorption of the total DOM pool at 330 nm.

The influence of MAAs on POM absorption at 330 nm was not as apparent as in the dissolved fraction, since UV-absorption was not correlated with particulate MAA concen-

tration (Fig. 4, inset). Removal of the detrital component from total POM absorption to yield phytoplankton absorption did not improve the relationship with MAA concentration. A pigment packaging effect of the MAAs within the phytoplankton cells (Moisan and Mitchell pers. comm.) may affect the absorption by MAAs within intact phytoplankton cells. Alternatively, incomplete extraction of MAAs could mask the effect of MAAs on UV absorption by POM. A recent investigation revealed that extraction in heated 20% aqueous methanol yielded significant ($\sim 10\times$) increases in the amount of MAAs in mixed populations of phytoplankton (Sommaruga and Garcia-Pichel 1999). This suggests that there may be two MAA classes within a cell: those that are easily extractable at low temperature in methanol (as in this study), and a second group requiring a more rigorous extraction procedure. This is also indicated by the differences between the suite of MAAs observed in the *L. polyedrum* culture (Vernet and Whitehead 1996) and those measured during the red tide (this study). The third possibility for the lack of a relationship between POM UV absorption and MAA concentration may be due to interference by other UV-absorbing components in the field that are not present, or exist in greatly reduced amounts, in laboratory studies. A similar mechanism may also be acting in the dissolved fraction since high MAA concentration does not consistently result in a visible shoulder in UV absorption. The MAA signal may be masked by high concentrations of other UV-absorbing dissolved components (colloidal organic matter and humic substances).

Our data indicate the presence of MAAs as part of the DOM pool in coastal waters, with concentrations varying from 3.43 to 111.40 nM. These are the first published measurements of MAAs in the dissolved form in natural samples. As expected, MAA-biosynthesizing phytoplankton appear to be the main source of MAAs to the water column, likely via cellular excretion or cell lysis and grazing. Understanding the sources and the removal mechanisms of MAAs is key to elucidating the effect these compounds and similar UV-absorbing compounds have on the underwater UV radiative field. The influence of dissolved MAAs on UV attenuation by DOM, as examined here, was disproportionately large in comparison to their relatively low concentrations. The resulting decrease in UV transmission through the water column has the potential of conferring added UV photoprotection to the plankton assemblage. Improvements in the extraction of dissolved MAA and MAA-like compounds and in our ability to chemically characterize these compounds will allow for further studies to investigate the role of dissolved MAAs and similar UV-absorbing compounds in UV absorption in the dissolved phase and their photoprotective role within phytoplankton.

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