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Phytoplankton as a temperate marine source of brominated methanes

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ABSTRACT

Bromoform ($CHBr_3$) has an important role in transporting bromine from the ocean to the atmosphere, and released bromine catalyses ozone depletion. In temperate ocean waters, a number of studies have observed or presumed $CHBr_3$ production. Here, we studied the ability of marine phytoplankton to produce $CHBr_3$ in cultures of temperate phytoplankton. A temperate marine diatom and a cyanobacterium, *Ditylum brightwellii* CCMP 358 and *Synechococcus* sp. CCMP 1334, respectively, were incubated at 24 °C and the concentrations of brominated methanes in the cultured samples were determined using purge and trap gas chromatograph–mass spectrometry. The axenic cultures of the diatom exhibited a remarkable rate of $CHBr_3$ production, ~200 nmol (g chlorophyll *a*)$^{-1}$ h^{-1}, which was several times higher than that for cold water diatoms reported previously. The cyanobacterium also produced $CHBr_3$, with a production rate of ~1 nmol (g chlorophyll *a*)$^{-1}$ h^{-1}, shows that two diverse phytoplankton can produce $CHBr_3$. Both $CHBr_2Cl$ and $CHBrCl_2$ were also produced in the *D. brightwellii* culture, but only $CHBr_2Cl$ was produced in the culture of the *Synechococcus* sp. An incubation experiment with $^{13}CHBr_3$ revealed that there was no $^{13}CHBr_3$ degradation (or the formation of $^{13}CHBr_2Cl$ and $^{13}CHBrCl_2$) in the cultures of these two phytoplankton, and these results suggested that both diatoms and cyanobacteria could produce chlorinated methanes. Our results suggest that brominated methanes such as $CHBr_3$ are produced by temperate phytoplankton and that phytoplankton is a significant source of $CHBr_3$ in the temperate open ocean.

1. Introduction

Short-lived bromomethanes such as bromoform ($CHBr_3$) have important roles in transporting bromine from the ocean to the atmosphere (Carpenter and Liss, 2000; Quack et al., 2004). $CHBr_3$ releases bromine into the troposphere and the stratosphere via photolysis, and released bromine catalyses ozone depletion (Read et al., 2008). In terms of the contribution of very short-lived substances to stratospheric bromine content, $CHBr_3$ and dibromomethane (CH_2Br_2) are thought to be the two most significant compounds (Aschmann and Sinnhuber, 2013). Observations of $CHBr_3$ distribution in the open ocean have shown that higher concentrations of $CHBr_3$ are present over tropical and warm ocean regions (e.g., Quack et al., 2004; Ziska et al., 2013). A modelling study showed that open oceanic emission of $CHBr_3$ made a notable contribution to the bromine distribution in the troposphere and that the global emission distribution of $CHBr_3$ classifies tropical and warm areas as significant sources of $CHBr_3$ (Liang et al., 2010; Ordóñez et al., 2012). In addition, the bottom-up emission estimate for $CHBr_3$ using the HalOcAt (Halocarbons in the Ocean and Atmosphere) database showed that hot spots for emissions of $CHBr_3$ were located in the equatorial region (Ziska et al., 2013).

As for the sources of $CHBr_3$, marine macroalgae have been suggested to generate $CHBr_3$ in many previous studies. For example, high concentrations of $CHBr_3$ have been observed in algae beds in the Eastern Arctic Ocean (Dyrssen and Fogelqvist, 1981); elevated levels of $CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$, CH_2Br_2, and $CHCl_3$ were reported in beds of brown seaweed *Laminaria digitata* in the west coast of Scotland (Nightingale et al., 1995); and $CHBr_3$ production was observed in temperate marine algae in the coastal east Atlantic (Carpenter and Liss, 2000). Macroalgae produce halogenated organic compounds (reviewed by La Barre et al., 2010) and release volatile halogenated organic compounds, such as $CHBr_3$ (e.g., the brown algae Laminariales (kelp), the red algae *Rhodymenia californica*, and green algae *Ulva* sp.: Manley et al., 1992; and the brown algae *Dictyosyphon foeniculaceus*: Laturnus, 1996; Carpenter and Liss, 2000). With respect to the production of $CHBr_3$ by microalgae phytoplankton, $CHBr_3$ production by cold water diatoms such as *Porosira glacialis* (CCMP 651, not axenic), *Nitzschia* sp. (Tokarczyk and Moore, 1994; Moore et al., 1996) and *Thalassiosira* sp. (measurement of bromocarbon production in cultures with and without antibiotic treatment, Hughes et al., 2013) and $CHBr_3$ production by natural assemblages of ice algae (Sturges et al., 1992) have been reported. In a culture study on temperate marine cyanobacteria, no production of either $CHBr_3$ or CH_2Br_2 was observed in the cultures of either *Prochlorococcus marinus* (CCMP 2389) or *Synechococcus* sp. (CCMP 2370) (Hughes et al., 2011). To date, only a limited number of marine phytoplankton from culture collections have been studied in regard to their production of bromocarbons.

It has been reported that di- and tri-halogenated methanes such as $CHBr_3$ may be produced indirectly from haloperoxidase activity,

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utilizing H_2O_2 as a substrate, in macroalgae, phytoplankton, fungi, and bacteria (Manley, 2002; Winter and Moore, 2009). With respect to the haloperoxidase activity in cyanobacteria, Johnson et al. (2011) extracted a protein with a capacity for bromoperoxidase activity from axenic cultures of *Synechococcus*. Recently, both vanadium-dependent bromoperoxidase activity and $CHBr_3$ production were observed in a culture of *Synechococcus* (strain CC9311), whereas no production of $CHBr_3$ was found in the cultures of a mutant strain of CC9311 in which the gene for bromoperoxidase was disrupted (Johnson et al., 2015). Hill and Manley (2009) reported that the release of reactive bromine from diatoms and reaction with dissolved organic matter in seawater may contribute to the production of $CHBr_3$ in the tropical ocean. The production of $CHBr_3$ in seawater in reactions involving bromoperoxidase and H_2O_2 has been reported; $CHBr_3$ was produced from the chemical reaction between dissolved organic matter in seawater and the hypobromous acid released by bromoperoxidase (Lin and Manley, 2012). However, in that report, the concentration of added H_2O_2 was about 1000-fold greater than that of H_2O_2 typically present in bulk seawater, though the levels of H_2O_2 could possibly be higher in the apoplast than in the surrounding seawater (Lin and Manley, 2012).

Reifenhäuser and Heumann (1992) measured the concentrations of $CHBr_3$, CH_2Br_2, $CHBr_2Cl$, and $CHBrCl_2$ in seawater near the Antarctic Peninsula, and found good correlations among different bromocarbons – e.g., the concentrations of $CHBr_3$ were associated with those of CH_2Br_2 – suggesting that these bromocarbons have a similar biological source in seawater. High concentrations of $CHBr_3$ were observed in coastal seawater of the Southern Ocean and ice algae were suggested to be the source of $CHBr_3$ in this region (Carpenter et al., 2007). Seasonal changes of $CHBr_3$ and CH_2Br_2 were observed in seawater in a coastal bay on the western Antarctic Peninsula, and the highest concentrations of $CHBr_3$ and CH_2Br_2 were found to correspond with microalgal bloom (Hughes et al., 2009). In coastal seawaters of the western Antarctic Peninsula, higher concentrations of $CHBr_3$ (mean: 122 pmol L^{-1}) were observed in seawater collected during the diatom bloom compared to those (mean: 42.9 pmol L^{-1}) in seawater collected under non-bloom conditions, whereas CH_2Br_2 concentrations were not significantly different between seawaters collected during the diatom bloom and those collected during non-bloom conditions (Hughes et al., 2012). With respect to the ratio of CH_2Br_2 to $CHBr_3$ in seawater in the northeast Atlantic and tropical eastern Atlantic Ocean, a lower ratio of CH_2Br_2 to $CHBr_3$ was observed in coastal areas close to seaweed sources, and the concentration ratio of CH_2Br_2 to $CHBr_3$ was found to be dependent on both the concentration and location (Carpenter et al., 2009). Hughes et al. (2009) showed that the ratio of CH_2Br_2 to $CHBr_3$ in seawater was also dependent on the microalgal bloom stage in a coastal bay on the western Antarctic Peninsula.

From the measurements of $CHBr_3$ distribution in the ocean, Quack et al. (2007) reported that the concentrations of $CHBr_3$ in seawater were not associated with high chlorophyll concentrations in upwelling areas in the temperate Atlantic Ocean (Quack et al., 2007). However, in contrast to the chlorophyll concentrations, it has been reported that the production of brominated gases such as $CHBr_3$ in the upwelling areas was significantly correlated with low concentrations of indicator pigments for diatoms or cyanobacteria (Quack et al., 2007). An association between $CHBr_3$ production and cyanobacterial blooms has also been reported, and the production of $CHBr_3$ has been suggested to result from cyanobacterial production in the Baltic Sea (Karlsson et al., 2008). In a related study, field research into the spatial and temporal trends of brominated compounds in seawater suggested that brominated compounds such as $CHBr_3$ could be released from the detritus derived from the *Trichodesmium* bloom in the coastal eastern Arabian Sea (Roy et al., 2011). In addition, it has been reported that the production of $CHBr_3$ and CH_2Br_2 may be attributable to phytoplankton species in the open ocean (Liu et al., 2013).

Studies on modelling $CHBr_3$ distribution in the ocean have assumed a relationship between $CHBr_3$ production and phytoplankton biomass (Hense and Quack, 2009; Palmer and Reason, 2009). In addition, phytoplankton marker pigments such as chlorophyll *b*, chlorophyll *c3*, fucoxanthin, diatoxanthin, pyrophaeophorbide *a* and zeaxanthin were related to the distribution of $CHBr_3$ based on the observation of halocarbons and phytoplankton pigments in surface seawater in the tropical North East Atlantic, including upwelling areas (Hepach et al., 2014). To date, however, direct evidence of the production of $CHBr_3$ by temperate marine phytoplankton has not been reported.

In this study, we describe the measurements of $CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$ and CH_2Br_2 concentrations in axenic cultures of a temperate marine diatom, *Ditylum brightwellii* CCMP 358, and a cyanobacterium, *Synechococcus* sp. CCMP 1334, and calculate the production rate of brominated methanes in culture. In the modern ocean, diatoms account for approximately 40% of the net primary production and are one of the main phytoplankton groups that play a major role in carbon cycling (Falkowski et al., 2004). *Synechococcus* is also an abundant representative of phytoplankton and contributes significantly to primary production in the ocean (Li, 1994; Zwirglmaier et al., 2008). Finally, in the case of $CHBr_3$, we also measured the degradation of this compound by the two phytoplankton by adding $^{13}CHBr_3$ to the cultures and measuring changes in its concentration.

2. Experimental

2.1. Phytoplankton strains and growth conditions

The temperate marine diatom (*D. brightwellii* CCMP 358, originating from the Gulf of Mexico) and unicellular marine cyanobacteria (*Synechococcus* sp. CCMP 1334, from the Sargasso Sea) were obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA (formerly the CCMP), Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA). According to the NCMA guidelines, suitable incubation temperatures for these two phytoplankton range from 22 °C to 26 °C. Glass culture vessels (conical flasks made of gas-tight borosilicate; Pyrex) sealed with ground glass caps (gas-tight borosilicate; Pyrex) containing autoclaved f/2 or f/2-Si media (1500 mL, prepared in 0.2 μm filtered, aged seawater) for the *D. brightwellii* or *Synechococcus* sp., respectively, were assembled (Guillard and Ryther, 1962). $NaHCO_3$ (0.6 g L^{-1}) was added to the cultures for *D. brightwellii*. The initial headspace in the vessels was approximately 840 mL. Following the inoculation with *D. brightwellii*, the culture was incubated at 24 ± 0.2 °C with a light/dark cycle of 14/10 h and a light intensity of 120 μmol photons m^{-2} s^{-1} provided by full-spectrum daylight via a Vita-Lite fluorescent lamp (Naturallighting.com, Dickinson, TX, USA). The cultures of the *Synechococcus* sp. were also incubated at 24 ± 0.2 °C, but the light/dark cycles (12/12 h) and light intensity (80 μmol photons m^{-2} s^{-1}) were slightly different. A flask containing only the medium (not inoculated with phytoplankton) was used as a control for each phytoplankton. Sterile techniques were used at all times and the cultures were handled on a clean bench (SC-13FAG; Dalton Corp., Tokyo, Japan).

2.2. Time-course experiment and measurement of brominated methanes in cultured samples

To obtain the time courses of brominated methane concentrations in the cultures of phytoplankton, the levels of $CHBr_3$, $CHBr_2Cl$, $CHBrCl_2$ and CH_2Br_2 were measured in the phytoplankton cultures for 32–35 days. Phytoplankton growth was monitored by measuring chlorophyll *a* (chl. *a*) during the culture period. For each species two replicate cultures were grown in separate flasks. At intermittent intervals (several days), aliquots of phytoplankton cultures were sampled from near the base of the glass culture vessels by a gas-tight glass syringe with a section of the Tygon tubing for brominated methanes analysis (100 mL) and pH and chl. *a* measurement (10 mL). At each sampling, we removed the cap of the glass culture vessel and performed the sampling within