

Release of reactive bromine and iodine from diatoms and its possible role in halogen transfer in polar and tropical oceans

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Abstract

An in situ incubation assay measuring the bromination and iodination of phenol red was developed to detect the release of reactive bromine and iodine (primarily hypobromous acid [HOBr] and hypoiodous acid [HOI], respectively) from a putative extracellular bromoperoxidase of marine diatoms. Six of 11 species showed significant release compared to controls. Polar species were particularly active, releasing 0.6–180 fmol HOBr cell⁻¹ h⁻¹ (0.04–1.8 μmol HOBr [mg total chlorophyll]⁻¹ h⁻¹; at the seawater bromide concentration, 840 μmol L⁻¹) and 1.9–271 fmol HOI cell⁻¹ h⁻¹ (0.02–2.7 μmol HOI [mg total chlorophyll]⁻¹ h⁻¹, at 100 μmol L⁻¹ iodide). *Porosira glacialis* consistently showed the highest rates of release. Several temperate diatoms, including *Achnanthes cf longipes*, known to have a bromide-sensitive peroxidase involved in stalk formation, and warm-water species also showed the ability to release reactive bromine and iodine. This release was influenced by light, temperature, bromide (and iodide) concentration, H₂O₂ concentration, and pH. The rate of HOBr release by polar diatoms was much greater than bromoform emissions measured by others from laboratory cultures and sea-ice algae in the field. This indicates that most of the HOBr released may react with dissolved organic matter (DOM) to form nonvolatile bromine organics. Some fraction of diatom-produced HOBr and HOI may also form volatile Br₂ and I₂, which could transfer to the polar troposphere. The reaction of diatom-released reactive bromine and iodine with seawater DOM may represent the major mechanism in the formation of oceanic polybromo- and polyiodo-methanes.

Marine algae (phytoplankton and seaweeds) are important intermediaries in the chemical transformation of seawater halides to volatile halocarbons. The volatile halocarbons are important vectors of halogen transfer from the oceans to the atmosphere. One class of these algal-produced volatile halocarbons, the atmospherically short-lived polyhalomethanes, are responsible for the transport of the vast majority of organo-bromine (Br), primarily as bromoform (CHBr₃), and for transport of a significant amount of organo-iodine (I), primarily as diiodomethane (CH₂I₂; Carpenter 2003; Butler et al. 2007; Saiz-Lopez et al. 2007). The oxidation of bromide and iodide and the subsequent bromination and iodination resulting in these compounds is catalyzed by algal bromoperoxidase (BrPO). The less-prevalent iodoperoxidase (IPO) utilizes iodide only.

Marine phytoplankton and sea-ice algae, especially diatoms, are known producers of CHBr₃ and CH₂I₂ (Moore et al. 1996; Sturges et al. 1997). BrPO and IPO have been detected in these diatoms (Moore et al. 1996). Bromoform released from sea-ice algae was for a time considered the major source of bromine responsible for the episodic arctic tropospheric ozone loss. However, it was later determined that the rate of CHBr₃ photolysis was too slow to account for the buildup of the reactive bromine species (Martinez et al. 1999). Subsequently, a nonbiological mechanism was deduced, which accounted for the large reactive bromine release (Foster et al. 2001; Kaleschke et al. 2004).

Coastal release of polybromo- and polyiodo-methanes is dominated by seaweeds, whereas oceanic production comes from phytoplankton, presumably diatoms, especially in equatorial regions (Carpenter 2003; Quack and Wallace 2003; Butler et al. 2007). Bromoform released from midlatitude and tropical oceans may be responsible for most of the tropospheric bromine in specific locations with high primary production (Quack et al. 2004). If the theoretical nonbiological marine sea-salt aerosol mechanism of reactive bromine production does not occur, then algal-produced CHBr₃ may be the major global source (Quack and Wallace 2003).

In this paper, we describe the significant release of hypobromous acid (HOBr) and hypoiodous acid (HOI) from polar, temperate, and warm-water diatoms into their surroundings. These reactive halogen compounds should equilibrate in aqueous solution with their respective molecular halogens (Br₂ and I₂) and trihalides (e.g., Br₃⁻¹ and I₃⁻¹). We refer to all of these compounds as reactive bromine or iodine.

Methods

Cultures and standard techniques—Diatoms were obtained from the Center for the Culture of Marine Phytoplankton (CCMP–Bigelow Laboratory for Ocean Sciences): *Porosira glacialis* (CCMP 651), *Nitzschia* sp. (CCMP 580), *Nitzschia cf promare* (CCMP 1116), *Stauroneis cf glacialis* (CCMP 1085), *Navicula* sp. (CCMP 544), *Amphora coffeaeformis* (CCMP 127), *Thalassiosira pseudonona* (CCMP 1335), *Phaeodactylum tricornutum* (CCMP 632), *Thalassiosira oceanica* (CCMP 1005), *Fragilaria pinnata* (CCMP 395), and *Achnanthes cf longipes* (CCMP

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101). *A. longipes*, *A. coffeaeformis*, *T. pseudonona*, and *P. tricorutum* are temperate species, *T. oceanica* and *F. pinnata* are subtropical (warm-water) species, and all other species are polar. All species were grown in f/2-enriched seawater media with a 12-h photoperiod at 4°C and 22.2 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (polar species), at 20°C and 22.2 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (temperate species), and at 25°C and 105 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (warm-water species). Chlorophyll (Chl) concentrations were determined for each species using the modified method of Arnon (1949). Cell counts were made using a hemocytometer.

In situ incubation assay—The in situ incubation assay for diatom BrPO catalyzed production and release of HOBr and HOI is based on the halogenation of phenol red (PR; phenolsulfonphthalein) to bromophenol blue (BPB; 3',3'',5',5''-tetrabromophenolsulfonphthalein) and iodo-phenol blue (IPB; 3',3'',5',5''-tetraiodophenolsulfonphthalein) in the presence of H_2O_2 . Although brominated in the presence of BrPO, it has been shown that PR cannot enter the active site (Tschirret-Guth and Butler 1994). This assay is a modification of that used for seaweeds (Wever et al. 1991). Phenol red and BPB were measured spectrophotometrically at 433 nm ($\epsilon = 19.7 \text{ mmol L}^{-1} \text{ cm}^{-1}$) and 592 nm ($\epsilon = 67.4 \text{ mmol L}^{-1} \text{ cm}^{-1}$), respectively. The molar extinction coefficient for IPB has not been previously reported, and the compound is not commercially available; therefore, it was produced and purified for this study (Hill 2008). The IPB was prepared by completely reacting 10 mL of 25 $\mu\text{mol L}^{-1}$ PR, 100 $\mu\text{mol L}^{-1}$ iodide, and 1.5 mmol L^{-1} H_2O_2 in commercial BrPO enzyme (from *Corallina officinalis*, Sigma-Aldrich). Absorbance readings were taken periodically to determine when all the PR had reacted. After the PR had completely reacted, the solution was 97% IPB, with the remaining 3% being putatively determined as monoiodophenol blue by thin-layer chromatography (TLC). IPB was purified by TLC: silica gel No. 6, ethyl acetate, methanol, 5 mol L^{-1} NH_4OH (60:30:10). The IPB fraction was isolated, eluted from the silica, and its absorption spectrum determined. The peak absorbance was at 600.9 nm, with $\epsilon = 47.4 \text{ mmol L}^{-1} \text{ cm}^{-1}$. The changes in PR, BPB, and IPB absorption maxima were used to determine the rate of the removal of PR by bromination (or iodination) and the rate of BPB (or IBP) production.

Diatoms were isolated ($\sim 1/8$ mL packed cell volume) by gentle centrifugation and added to a 50-mL rectangular plastic culture flask containing 10 mL of 100 mmol L^{-1} phosphate buffer (pH 6.5) with 25 $\mu\text{mol L}^{-1}$ PR, either I^- (100 $\mu\text{mol L}^{-1}$) or Br^- (840 $\mu\text{mol L}^{-1}$; average seawater concentration), and 35‰ NaCl (average seawater salinity). H_2O_2 was added to initiate the reaction. The standard (and optimal) H_2O_2 concentration used varied with each diatom species. Controls with reactants but without cells were run at H_2O_2 concentrations of 0.24 and 15 mmol L^{-1} . Controls with cells only and no PR or H_2O_2 were run to discount any spectral interference. A control with no H_2O_2 was also run for each variable tested (e.g., pH, halide concentration, growth phase). The culture flasks were gently shaken continuously on a mechanical shaker at 24°C (room

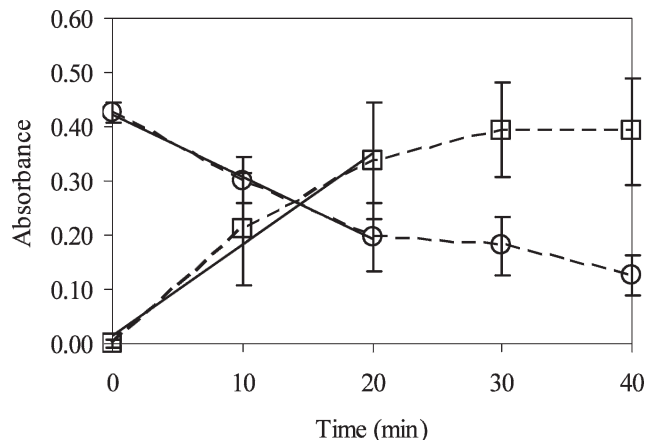


Fig. 1. Disappearance of PR (circles) and formation of BPB (squares) as measured by absorbance at 433 nm and 592 nm, respectively, during in situ incubation BrPO assay with *P. glacialis*. Regression line for first 20 min; $r^2 = 0.99$ and 0.98 for PR and BPB, respectively ($n = 3$).

temperature) and under dim room lighting (cool white fluorescent, 0.6 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Microscopic examination of the cells after the incubation period revealed no cell breakage. A 1.5-mL sample was taken with a 3-mL plastic syringe every 5–20 min. Cells were removed with a syringe filter (0.22- μm -pore diameter cellulose filter), and the filtrate was analyzed spectrophotometrically. Monochlorodimedon (MCD; 50 $\mu\text{mol L}^{-1}$), the standard substrate used to determine BrPO activity (Everett et al. 1990), replaced PR in the standard assay to verify the enzyme was a BrPO.

For all assays, cells were counted using a hemocytometer, and the rate of PR removal and bromination (or iodination) was normalized on a per-cell basis. The rate was determined from the linear portion of the graph based on no less than three data points (see Fig. 1 as an example). The surface area of each diatom species was determined by microscopically measuring cell dimensions, and BrPO activity was also calculated per surface area (Sun and Liu 2003). Student's *t*-tests were used to determine if the experimental results were significantly different from the control ($n = 3$ –9).

P. glacialis, a diatom species known to produce bromoform (Moore et al. 1996) and exhibiting the highest BrPO activity (rate of PR halogenation), was used to determine the optimal conditions for the in situ assay by testing its BrPO activity at varying pH levels (5–8), bromide concentrations (0–1500 $\mu\text{mol L}^{-1}$), iodide concentrations (0–200 $\mu\text{mol L}^{-1}$), H_2O_2 concentrations (0–3 mmol L^{-1}), and temperatures (room temperature and 4°C). Only one factor was varied at a time; all other factors were kept at their determined optimum. Because algae can metabolically produce H_2O_2 , especially as a by-product of photosynthesis, the in situ BrPO assay was run as described with and without the addition of H_2O_2 both in the light and in the dark. The in situ BrPO assay was also performed on *P. glacialis* cells from log and stationary phases of growth.

To determine whether bacteria played a role in BrPO activity, bacterial counts were conducted on *P. glacialis*

cells cultured under standard culture conditions and cells cultured with the combined antibiotics penicillin G (250 mg L⁻¹), streptomycin (125 mg L⁻¹), and chloramphenicol (25 mg L⁻¹) (Howshaw and Rosowski 1973). To enumerate the bacteria present, 1 mL of each culture was mixed with 100 μ L acridine orange (Hobbie et al. 1977). Bacterial counts were taken using fluorescence microscopy. The standard PR in situ assay was run using cells from each culture after counts were taken. The difference in BrPO activity was compared to the difference in bacterial counts between the two cultures.

Potential inhibition of the incubation assay by sodium azide, ethylenediaminetetraacetic acid (EDTA), and exposure to high H₂O₂ concentration were tested in an attempt to determine whether *P. glacialis* has vanadium BrPO (V-BrPO) or iron (Fe)-heme BrPO. Sodium azide has been reported to inhibit Fe-heme BrPO (Moore et al. 1996), while EDTA has been reported to inactivate vanadium enzymes at low pH (Everett et al. 1990). A Student's *t*-test was used to compare the activity between BrPO treated with potential inhibitors and the standard assay. Sodium azide (1.0 mmol L⁻¹) was added to the standard assay solution with PR, bromide, and H₂O₂. EDTA (1.0 mmol L⁻¹) was added to a pH 5.5 solution containing the standard concentrations of PR, bromide, and H₂O₂. The resulting activity was compared to the results from a standard assay run at pH 6.5 and pH 5.5. Fe-heme BrPO is permanently inhibited by exposure to high concentrations of H₂O₂ (Soedjak et al. 1995). To test whether inhibition of *P. glacialis* BrPO activity with high H₂O₂ concentration was permanent, cells were treated with an inhibitory concentration (2 mmol L⁻¹) of H₂O₂ in the phosphate assay buffer with NaCl (minus PR, at pH 6.5) for 20 min, followed by a thorough rinse with this solution minus H₂O₂. The cells were then tested using the standard incubation assay with optimal H₂O₂ (0.24 mmol L⁻¹) and compared with untreated cells to determine if the permanent inhibition occurred.

In situ BrPO activity from other diatom species—After the optimum conditions for BrPO activity were determined using *P. glacialis*, other diatom species were screened for BrPO activity using the standard in situ PR assay at room temperature. The bromide optimum concentration for *P. glacialis* was used along with an [H₂O₂] ranging from 0.1 to 5.0 mmol L⁻¹. Only the starting and ending absorbances at 433 nm were compared during the initial screening. If BrPO activity was detected, full assays were run with that species at the optimum [H₂O₂], as determined by the screening. If no activity was detected, the same screening process was done using iodide. If activity was detected with iodide, the PR assay was performed, using iodide, at the optimal H₂O₂ concentrations, as determined by the screening. Each species' BrPO activity (rate of PR halogenation) was normalized per cell, per cm² cell surface area, and per mg Chl. For *A. longipes*, *Navicula* sp., and *T. pseudonana*, the effect of varying H₂O₂ concentrations was determined as described in the previous section for *P. glacialis*.

Dialysis membrane experiments—A dialysis membrane bag (MWCO 100 Daltons) was used to demonstrate that PR (MW = 354.4 Daltons) halogenation occurred outside the cell. It was first verified that PR could not pass through the membrane. In the course of this determination it was found that a small amount of PR could adsorb to the membrane surface. *P. glacialis* cells in 100 mmol L⁻¹ phosphate buffer (pH 6.5) with 35‰ NaCl, Br⁻ (840 μ mol L⁻¹) or I⁻ (100 μ mol L⁻¹), and H₂O₂ (0.24 mmol L⁻¹) were sealed in the dialysis bag and placed into the identical buffered solution that lacked cells and contained PR (25 μ mol L⁻¹). The change in absorbance of PR and BPB (or IPB) was measured in the external solution. The changes in concentrations between the experimental and control assays (no cells) were compared using Student's *t*-test. The released HOBr and HOI from the cell would presumably be released from an external BrPO, not an internal one, because these reactive halogens would deleteriously react with cellular constituents.

Genomic comparisons—The genome of *T. pseudonana* is known, so a National Center for Biotechnology Information (NCBI) protein search and a Basic Local Alignment Search Tool (BLAST) search (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) were performed (07 December 2007) to determine if the region encoding BrPO could be identified. Searches were run using V-BrPO sequences from the fungus *Curvularia inaequalis* (accession No. 1VNH_A), the brown algae *Fucus distichus* (accession No. AF053411.1), the red algae *Corallina pilulifera* (accession No. D87657.1), and the bacterium *Streptomyces violaceus* (accession No. AAB84315). Joint Genome Institute (JGI; <http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Thaps3&advanced=1>) protein vs. translated nucleotide and nucleotide vs. nucleotide BLAST searches were run using the BrPO sequences listed above against *T. pseudonana* using the default values. A positive control with Rubisco small subunit was also run against *T. pseudonana*.

Results

In situ incubation assay using P. glacialis—PR was brominated and iodinated by incubated *P. glacialis*, resulting in the formation of BPB and IPB (Fig. 1). Neither BPB or IPB formation nor PR disappearance was detected at H₂O₂ concentrations of 0.24 and 15 mmol L⁻¹, when cells were not added (Table 1). Also, there was no spectral interference due to release of cell dissolved organic matter (DOM) over the incubation period. The optimum pH for in situ bromination of PR was 6.5 (Fig. 2). The optimum iodide concentration for in situ iodination of PR was 100 μ mol L⁻¹, and higher concentrations of iodide inhibited BrPO activity (Fig. 3A). High bromide concentrations were not inhibitory, but rather BrPO activity displayed saturation kinetics leveling off at approximately 840 μ mol L⁻¹ bromide (Fig. 3B). The optimum H₂O₂ concentration for bromination and iodination was between 0.2 and 0.3 mmol L⁻¹ (Fig. 4). There was low inherent halogenation of PR under room lighting and in the dark when H₂O₂ was not added (Table 1).

Table 1. The effect of various treatments on the in situ BrPO activity of *P. glacialis*.

Assay conditions	Relative BrPO activity (%)	<i>p</i> -value
Optimal conditions	100	—
4°C	77	0.10
pH 5.5	57	0.25
+ Antibiotics	77	0.045*
+ EDTA†	57(100)‡	0.13(0.99)‡
+ Azide	-3	0.001*
No added H ₂ O ₂ ; light§	24	0.006*
No added H ₂ O ₂ ; dark	11(48)	0.006*(0.295)
Pretreatment with 2 mmol L ⁻¹ H ₂ O ₂	137	0.27
No cells (reactants only)¶	1.8, 3.6	0.035*,0.032*

* Denotes significant difference from optimal conditions at $p < 0.05$.

† Assay run at pH 5.5. EDTA, ethylenediaminetetraacetic acid.

‡ Comparison with pH 5.5 assay.

§ Room lighting, 0.6 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

|| Comparison with "no added H₂O₂; light" assay.

¶ At H₂O₂ 0.24 and 15 mol L⁻¹, respectively.

The standard enzymatic BrPO assay substrate MCD (50 $\mu\text{mol L}^{-1}$) was brominated using the standard in situ assay at a rate of 315.7 $\text{fmol cell}^{-1} \text{h}^{-1}$. Calculations based on the halogenation of PR at optimum conditions with *P. glacialis* showed release rates of 179.3 $\text{fmol HOBr cell}^{-1} \text{h}^{-1}$, 7.6 nmol HOBr $\text{cm}^{-2} \text{h}^{-1}$, and 1.8 $\mu\text{mol HOBr (mg total Chl)}^{-1} \text{h}^{-1}$ and 271.2 $\text{fmol HOI cell}^{-1} \text{h}^{-1}$, 11.5 nmol HOI $\text{cm}^{-2} \text{h}^{-1}$, and 2.7 $\mu\text{mol HOI (mg total Chl)}^{-1} \text{h}^{-1}$.

There was no significant difference in BrPO activity between *P. glacialis* in stationary or exponential growth ($n = 3$, $p = 0.71$). A 47% decline in activity occurred for assays performed at 4°C compared to those run at room temperature (24°C; Table 1). There was a significant difference in the number of bacteria in cultures between those treated with antibiotics (1.4 bacteria cell^{-1}) and those not treated (7.3 bacteria cell^{-1}) (t -test: $df = 9$, $p = 0.001$) and a significant difference in BrPO activity between cultures treated with antibiotics and those not treated (t -test: $df = 3$, $p = 0.045$) (Table 1). Cells treated with antibiotics showed a 23% reduction in BrPO activity,

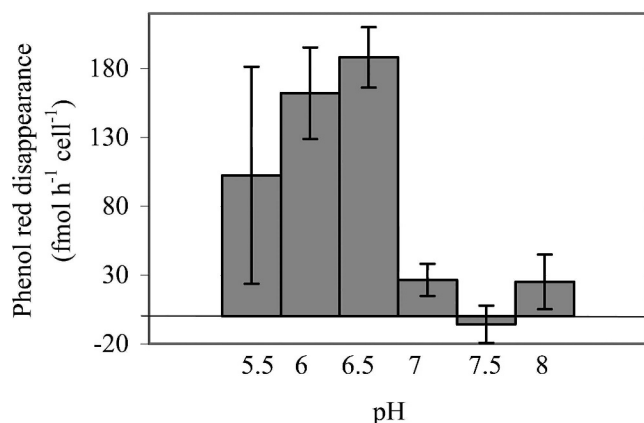


Fig. 2. *P. glacialis* BrPO activity (bromination of PR) at varying pH ($[\text{Br}^-] = 840 \mu\text{mol L}^{-1}$, $[\text{H}_2\text{O}_2] = 0.24 \text{mmol L}^{-1}$).

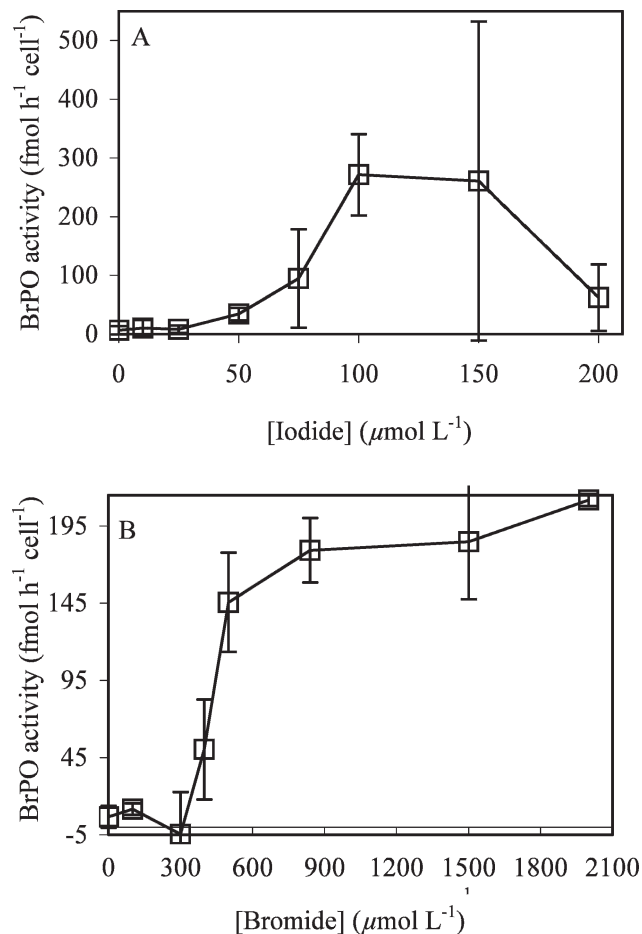


Fig. 3. *P. glacialis* BrPO activity at varying (A) iodide concentrations and (B) bromide concentrations (pH 6.5, $[\text{H}_2\text{O}_2] = 0.24 \text{mmol L}^{-1}$).

despite a bacterial reduction of 80%. The *P. glacialis* cultures treated with antibiotics grew much more slowly than did antibiotic-free cultures.

The effects of various treatments designed to inhibit the in situ BrPO assay of *P. glacialis* are shown in Table 1. Whereas the addition of sodium azide resulted in the complete loss of activity, prolonged exposure of the cells with a high concentration of H₂O₂ (2 mmol L⁻¹) prior to the assay did not significantly change the bromination of PR when compared to the control. The addition of EDTA to the assay solution at pH 5.5 did not alter the activity of BrPO compared to the assay run at the same pH minus EDTA. The decline in activity when compared to the pH 6.5 control was due to the pH (Fig. 2).

In situ BrPO activity from other diatom species—The in situ BrPO activity measured from incubations of other species is shown in Table 2. Most of the polar species demonstrated activity. Most of the temperate and tropical species showed little or no activity. A notable exception was *A. longipes*. Optimum H₂O₂ concentrations for the in situ BrPO assay varied with species, and when rates were compared at 0.24 mmol L⁻¹, the optimum for *P. glacialis* and also the lowest optimum found for any of the diatoms

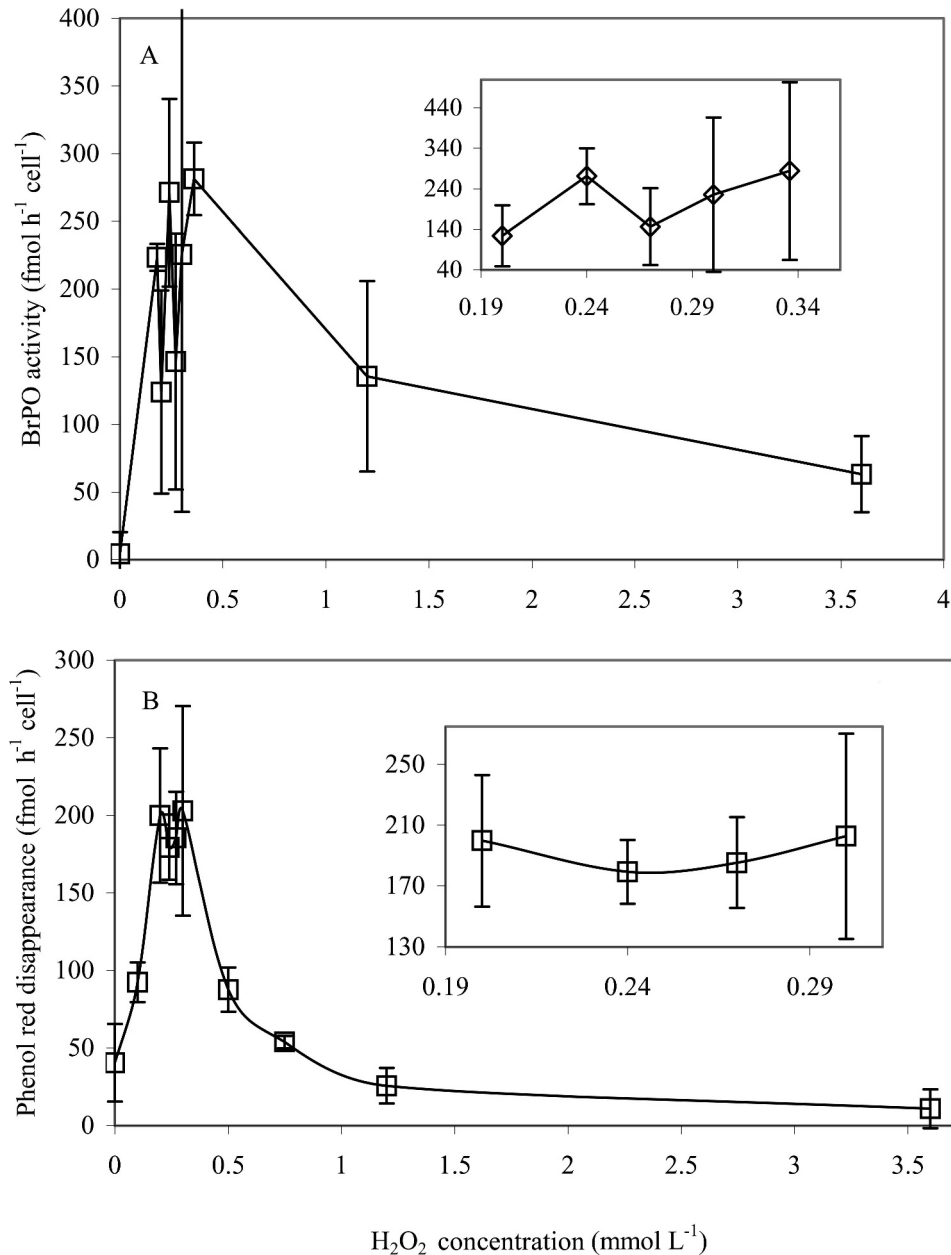


Fig. 4. *P. glacialis* BrPO activity at varying H₂O₂ concentrations. (A) Iodination of PR ([I⁻¹] = 100 μ mol L⁻¹; inset = activity between 0.2 and 0.4 mmol L⁻¹ H₂O₂). (B) Bromination of PR ([Br⁻¹] = 840 μ mol L⁻¹; inset = activity between 0.2 and 0.3 mmol L⁻¹ H₂O₂).

examined, most species did not show significant activity (Table 2).

Dialysis membrane experiments—There was a significant reduction in PR concentration due to bromination ($p = 0.10$) and iodination ($p = 0.05$) in the solution outside the dialysis bag between the experimental trials (*P. glacialis* cells present inside the dialysis bag) and the control (no cells; Table 3). The formation of BPB and IPB, respectively, occurred in the experimental series and not the controls. The decline in PR with the controls was found to be due to adsorption of PR on the outer membrane surface.

Genomic comparisons—No matches were found for any BLAST search done in NCBI or JGI for proteins or nucleotides that would encode BrPO. The positive control with Rubisco small subunit did match *T. pseudonana* (Loci: EF067921, DQ115007).

Discussion

In situ BrPO activity with *P. glacialis*—An in situ BrPO assay for seaweeds was first developed by Wever et al. (1991); in this study, whole thalli were exposed to PR in the presence of the added substrates H₂O₂ and bromide in

Table 2. Comparison of BrPO iodination (I) and bromination (Br) activity at each species' optimal H₂O₂ concentration and at 0.24 mmol L⁻¹ H₂O₂. P, polar species; T, temperate species; W, warm-water species; ns, not significant from control; nd, not determined; Optimum [H₂O₂] for species with no significant activity are concentrations that appeared to have activity during initial screening.

Species	Optimum H ₂ O ₂	fmol cell ⁻¹ h ⁻¹		nmol cm ⁻² h ⁻¹		μmol (mg total Chl) ⁻¹ h ⁻¹		fmol cell ⁻¹ h ⁻¹ at 0.24 mmol L ⁻¹ H ₂ O ₂	
		I	Br	I	Br	I	Br	I	Br
<i>Porosira glacialis</i> (P)	0.24	271±69	179±21	11.5±2.9	7.6±0.9	2.7±0.7	1.8±0.2	271±69	179±21
<i>Navicula</i> sp. (P)	15.0	16.2±7.0	26.4±4.7	1.0±0.5	1.7±0.3	0.16±0.07	0.64±0.11	nd	0.43*
<i>Nitzschia</i> sp. (P)	0.24	4.6±1.0	0.61±0.4	0.36±0.12	0.07±0.05	0.28±0.06	0.04±0.02	4.6±1.0	0.61±0.4
<i>Stauroneis glacialis</i> (P)	5.0	1.9±1.2	ns	0.05±0.03	ns	0.02±0.01	ns	0.09*	nd
<i>Nitzschia promare</i> (P)	5.0	ns	nd	ns	nd	ns	nd	ns	nd
<i>Achnanthes longipes</i> (T)	2.0	19±12	5.2±4.4	0.39±0.25	0.11±0.09	0.34±0.22	0.09±0.08	0	nd
<i>Thalassiosira pseudonana</i> (T)	10.0	nd	1.0±0.2	nd	0.98±0.2	nd	0.33±0.07	nd	ns
<i>Amphora coffeaeformis</i> (T)	0.24	nd	ns	nd	ns	nd	ns	nd	ns
<i>Phaeodactylum tricoratum</i> (T)	2.0	ns	nd	ns	nd	ns	nd	ns	nd
<i>Thalassiosira oceanica</i> (W)	5.0	nd	1.7±1.6	nd	0.56±0.53	nd	0.22±0.21	nd	0.08*
<i>Fragilaria pinnata</i> (W)	0.24	ns	nd	ns	nd	ns	nd	ns	nd

* Negative value from assay, value determined by interpolating trend line.

Table 3. The halogenation of phenol red separated from *P. glacialis* by a dialysis membrane.

	Phenol red disappearance (nmol min ⁻¹)	
	Bromination	Iodination
Experimental	18.1±17.2	10.7±6.2
Control*	8.7±3.9	5.1±0.0
<i>p</i> -value	0.10	0.05

* No bromophenol blue (BPB) or iodophenol blue (IPB) formation was detected in the outside solution containing PR; declines in PR are due to surface adsorption to membrane.

seawater. The authors concluded that the bromination of PR leading to the formation of BPB was due to release of HOBr from BrPO located externally on the thallus. Subsequently, it was shown with purified V-BrPO from a marine brown alga that PR does not enter the active site but is brominated via HOBr released from the enzyme (Tschirret-Guth and Butler 1994). Clearly the results of the dialysis experiment described herein demonstrate the release of HOBr and HOI from *P. glacialis* into the surrounding solution because of the halogenation of PR on the other side of the dialysis membrane (Table 3). This is the first report of such a release from a phytoplankton species.

Four halogenations of PR are required to form BPB or IPB. If PR was halogenated rapidly to the tetrahalo-compound, the ratio of PR disappearance rate to BPB or IPB formation rate would approach 1. If all the intermediates were formed at equal amounts, the ratio would be 4:1. Using the commercially available purified algal V-BrPO (*C. officinalis*), the PR:BPB rate ratio we determined was 1.4, indicating that multiple brominations occur rapidly in succession, approaching the 1:1 ratio. The PR:BPB ratio from the *P. glacialis* assay was 2.1; the PR:IPB ratio was 0.65. Theoretically a ratio of less than 1 should not occur; however, if there was significant spectral overlap with an iodinated intermediate and PR, such a ratio could result. It is therefore possible that the iodination rates are underestimated.

We attribute the BrPO activity to the diatoms rather than bacteria because (1) the number of bacterial cells per diatom cell in the nonaxenic cultures was extremely low, especially in the cultures of polar species; (2) there was no difference in halogenation rates between *P. glacialis* cultures in exponential and stationary phase, whereas the later phase showed an increase in bacterial numbers; (3) calculated rates of release based on bacterial cell surface area are extremely high and unrealistic (>600 μmol cm⁻² h⁻¹); and (4) *P. glacialis* cultured in the presence of antibiotics reduced the number of bacteria by 80%, compared to a culture without antibiotics, with only a 22% reduction in PR halogenation. It has been reported that the BrPO activity (units mg⁻¹ of BrPO enzyme) from the freshwater bacterium *Pseudomonas aureofaciens* was at least four orders of magnitude lower than the activity of algal BrPOs (van Pee and Lingens 1985). If the bacteria in the diatom cultures were contributing to the bromination of PR, it would likely be at a very low level compared to the

diatom contribution. Growth of *P. glacialis* in antibiotics resulted in very low growth compared to the growth of those that were untreated, and at times the cultures failed. The lowered BrPO activity of the treated cells may reflect a physiological stress.

We cannot state categorically that these reactive halogens come from a BrPO located outside the cell. The relatively high surface to volume ratio of these cells compared to a seaweed thallus could facilitate their release from inside the cell. If these reactive halogens were generated by an intercellular BrPO, however, they most likely would react with cellular constituents with possible deleterious consequences. We propose that the origin of the released HOBr and HOI by diatoms is from an extracellular BrPO, residing in the apoplast, the space between the cell membrane and silicified frustule. The process of stalk assembly and adhesion by the temperate benthic diatom *A. longipes* requires bromide, and the oxidative phenolic cross-linking that occurs is thought to be catalyzed by an extracellular peroxidase, possibly a BrPO (Johnson et al. 1995; Vreeland and Epstein 1996). This species did test positive for the bromination and iodination of PR using the in situ assay (Table 2), thus strongly indicating the presence of an extracellular BrPO. Extracellular BrPO and IPO have been identified in several seaweed species (Krenn et al. 1989; Kupper et al. 1998).

We found that the optimum pH for the bromination and iodination of PR by diatoms was 6.5, which is indicative of most algal haloperoxidases. Algae and plant cells maintain an acidic pH (~6) in the apoplast to facilitate the uptake of nutrient anions (Briskin 1990).

The in situ iodination rate by *P. glacialis* was 23 times higher than the bromination rates at 100 $\mu\text{mol L}^{-1}$ iodide and bromide, reflecting the higher oxidation potential of iodide. The average oceanic bromide concentration is 840 $\mu\text{mol L}^{-1}$, which is close to the BrPO maximum activity range determined for *P. glacialis* (Fig. 3B). Seawater iodide concentrations vary widely being between 3 and 300 nmol L^{-1} (Luther et al. 1988; Kupper et al. 1998). The optimum iodide concentration for PR iodination by *P. glacialis* was considerably higher, at 100 $\mu\text{mol L}^{-1}$ (Fig. 3A). Assuming a proportional drop in iodination with a drop in iodide concentration from 100 to 0.1 $\mu\text{mol L}^{-1}$, the ratio of in situ iodination to bromination ratio in seawater would be 6×10^{-3} , indicating that the brominating activity of the diatom BrPO dominates the iodinating activity in the marine environment.

The iodinating activity of *P. glacialis* was inhibited at high iodide concentrations ($>150 \mu\text{mol L}^{-1}$), and the activity at 150 $\mu\text{mol L}^{-1}$ was highly variable. In contrast to iodide, high concentrations of bromide were not inhibitory, but rather in situ BrPO activity followed saturation kinetics (Fig. 3B). The inhibition that occurred at high iodide concentrations could be caused by a suicide inhibition of the enzyme. Suicide inhibition is an irreversible inhibition when an enzyme product or intermediate permanently inactivates the active site. Iodination of vulnerable amino acid residues (i.e., tyrosine, phenylalanine, tryptophan) in the active site by HOI present in high concentrations may have occurred. Previous studies of

vanadium haloperoxidases from the kelp *Laminaria* found no inhibition with high iodide concentrations and found a K_m value of between 2.7 and 4.3 mmol L^{-1} (Almeida et al. 2001). These concentrations are considerably higher than the *P. glacialis* optimum.

It is also possible that at high iodide concentrations more HOI is produced but less HOI is released because a disproportionate amount was taken up into the cell as a result of multiphasic uptake. An extracellular haloperoxidase has been implicated in the accumulation of iodine in certain brown seaweeds (kelps), one that oxidizes iodide to HOI, a proposed bioavailable form (Kupper et al. 1998). The BrPO activity of *P. glacialis* that we measured was an order of magnitude higher than the other diatoms examined thus far, and this species has been shown to take up iodide at much greater rates than do other diatoms (De la Cuesta 1996). In general, diatoms are known to accumulate iodine (Vinogradov 1953), and it is intriguing to speculate that an extracellular BrPO, which can oxidize both bromide and iodide, might also be involved in iodine uptake.

The release of HOBr and HOI from an extracellular BrPO of marine diatoms may also minimize attack of pathogenic marine bacteria and viruses and may protect the cell from harmful H_2O_2 (Manley and Barbero 2001; Kupper et al. 2002). The BrPO-mediated reduction of H_2O_2 relies on a supply of electrons from bromide, the concentration of which is inexhaustible, rather than on diverting electrons from primary metabolic processes such as photosynthesis and mitochondrial respiration. This is particularly significant if this BrPO is outside the cell membrane.

Our in situ assays utilized H_2O_2 concentrations that are much greater than the concentration range for H_2O_2 in seawater (~20–200 nmol L^{-1} ; Miller and Kester 1994). Marine algae produce H_2O_2 via the Mehler reaction of photosynthesis (Manley and Barbero 2001) and by nonphotosynthetic metabolic processes, both of which can release H_2O_2 to their surroundings (10–10⁴ $\text{fmol cell}^{-1} \text{h}^{-1}$; Twiner and Trick 2000). Seaweed and higher plants are also known to contain peroxide-generating systems within the apoplast to deter pathogenic and parasitic attack (Kupper et al. 2002). Hydrogen peroxide concentrations in the diatom apoplast are probably much higher than in the surrounding seawater because of these production mechanisms. The low activity measured under room light when no H_2O_2 was added to the assay mixture most likely occurred because of H_2O_2 produced from the Mehler reaction (Table 1). Even lower activity occurred in the dark without added H_2O_2 .

The optimum H_2O_2 concentration for iodination and bromination activity of *P. glacialis* was 0.24 mmol L^{-1} , and high concentrations were inhibitory (Fig. 4). Almeida et al. (2001) found the K_m for V-BrPO from kelp to be between 0.13 and 0.27 $\text{mmol L}^{-1} \text{H}_2\text{O}_2$, for pH 6.1 and 6.7, respectively. They did not find high H_2O_2 concentrations to be inhibitory; however, they only tested up to 1.2 mmol L^{-1} . Everett et al. (1990) found the K_m for V-BrPO isolated from the brown seaweed *A. nodosum* to be 0.11 $\text{mmol L}^{-1} \text{H}_2\text{O}_2$ for the bromination of MCD and 0.09 mmol L^{-1}

H₂O₂ for the halide-assisted disproportionation of H₂O₂ to form singlet oxygen. When MCD is a substrate, its bromination (within the active site) competes with dioxygen formation. At pH 6.5, Everett et al. (1990) found that increasing the H₂O₂ concentration led to a decrease in the bromination of MCD and an increase in dioxygen formation. It is possible that the inhibition of the in situ BrPO assay at high H₂O₂ concentrations was due to more dioxygen formation, resulting in less HOBr or HOI being released to halogenate PR.

Reversible inhibition of V-BrPO activity at high concentrations of H₂O₂ is characteristic of halide-assisted V-BrPO disproportionation of H₂O₂ (Everett et al. 1990). After rinsing cells treated with 2 mmol L⁻¹ H₂O₂, a concentration found to be inhibitory (Fig. 4), the in situ BrPO activity was actually greater than that observed for the control (0.24 mmol L⁻¹; Table 1). This increase in activity was insignificant compared to the control and may have been due to residual H₂O₂ remaining in the apoplast. Whereas Fe-heme BrPO is permanently inhibited by high H₂O₂ concentrations, V-BrPOs are not (Soedjak et al. 1995). This result indicates that *P. glacialis* BrPO has a vanadium cofactor.

Azide was reported to inhibit only Fe-heme enzymes (Moore et al. 1996); however, we found that it inhibited commercially available V-BrPO enzyme (*C. officinalis*; Hill 2008). Azide is, therefore, not a reliable test for determining the BrPO cofactor. The addition of azide to the in situ assay mixture eliminated BrPO activity (Table 1). In addition to inhibiting both types of BrPO, azide is a well-known inhibitor of respiration and photosynthesis, and it has been shown to inhibit bromoform production from ice algae (Cota and Sturges 1997). Its inhibition of respiration is via the terminal oxidase, and its inhibition of photosynthesis is due to a buildup of H₂O₂ from the inhibition of catalase (a heme-containing enzyme; Forti and Gerola 1977). Clearly, in our assay any buildup of H₂O₂ would be negligible. The most direct explanation is that all peroxidases, including V-BrPO, were inhibited.

Treatment with EDTA at pH 5.5 did not reduce activity compared to no treatment with EDTA at the same pH, which has been seen to occur with purified V-BrPO at this or lower pH levels (Murphy et al. 2000; Almeida et al. 2001). A reduction in activity is due to the removal of vanadium from the protein. Almeida et al. (2001) found that different V-BrPOs isolated from the kelp *Laminaria* showed varying degrees and ease of inhibition with EDTA. It is possible that the pH used in the diatom incubation assay was not low enough or that the EDTA exposure time was not long enough to cause inhibition of *P. glacialis* BrPO activity. The result does not explicitly show that the enzyme is a Fe-heme BrPO rather than a V-BrPO.

In situ BrPO activity from other diatom species—*P. glacialis* had the highest activity of the diatoms examined, and all but one of the polar species showed significant activity (Table 2). The number of species tested is not large enough to allow general conclusions to be drawn. The BrPO activity of *P. glacialis* was not inhibited at the assay temperature (24°C) as compared to the activity at 4°C, its

optimum growth temperature (Table 1). It is assumed that the in situ BrPO activity for the other species was neither inhibited nor enhanced at this temperature.

Moore et al. (1996) isolated a BrPO from the same strain of *Nitzschia* sp. used in our study and an IPO from a different strain of *Navicula* sp. (CCMP 545; this strain was no longer available and we used CCMP 544 instead). We found that both species showed brominating and iodinating activity, indicating the presence of an extracellular BrPO in both (Table 2). Based on the in situ incubation assays, it appears that *S. glacialis* contains an IPO. *A. longipes*, *T. pseudonana*, and *T. oceanica* tested positive for BrPO in our assay, the latter being a warm-water strain. Our data supports the finding of a bromide-sensitive external peroxidase active in stalk formation of *A. longipes* (Johnson et al. 1995; Vreeland and Epstein 1996). The finding of activity in *T. oceanica* is significant in the context of bromoform production in warm water (see below). The widely different H₂O₂ concentration optima for the different species may reflect the presence of an alternative H₂O₂-degrading mechanism (those with high H₂O₂ optimum) or a lessened inherent H₂O₂ production mechanism (those with low H₂O₂ optimum).

T. pseudonana and *T. oceanica* had similar in situ BrPO activity (Table 2). The genomic comparison of *T. pseudonana* to V-BrPO from several algal sources failed to show a match. It is possible that the *T. pseudonana* genome is not complete and that there has been too much divergence between this diatom and other algal species. It is also possible that the gene for V-BrPO is not present and that Fe-heme BrPO is the enzyme present.

The environmental fate of released reactive bromine and iodine—The bromination and iodination of PR in our experiments clearly show the release of HOBr and HOI from six out of the 11 species examined. Most of the HOBr and HOI released by diatoms into the environment will react with DOM to form polybromo- and polyiodo-methanes in a process that is similar to the production of trihalomethanes (which include CHCl₃, CHBr₃, CHCl₂Br, and CHClBr₂) as by-products of drinking water disinfection using chlorination and ozonation (Wever et al. 1991). Bromoperoxidase-catalyzed production of CHBr₃ from seawater DOM has been measured (S. L. Manley and C. Lin unpubl.).

The same strains of *P. glacialis*, *Nitzschia* sp., and *N. promare* (formerly *N. arctica*) have been shown to produce CH₂Br₂ and CHBr₃ as the predominant products (Moore et al. 1996). The different strain of *Navicula* sp. (CCMP 545) produced CH₂I₂ and CH₂ClI only. Our assay of BrPO did not detect significant activity with *N. promare*; however, it displayed very low CHBr₃ and CH₂Br₂ production compared to the other species (Moore et al. 1996). The major polybromomethanes produced from marine algae are CHBr₃ and CH₂Br₂, with CHBr₃ to CH₂Br₂ ratios ranging from 1 to 68 for diatoms, with low light yielding higher ratios (Moore et al. 1996). Any chlorinated derivatives are a product of chloride exchange in seawater (Class and Ballschmiter 1988). Sea-ice algae, primarily diatoms, were found to produce much more

CHBr_3 than CH_2Br_2 (Sturges et al. 1992, 1997). Bromoform production by *P. glacialis* in culture was the highest among the diatoms examined, at $0.44 \text{ fmol Br cell}^{-1} \text{ h}^{-1}$ (Moore et al. 1996). The amount of HOBr released by this species under optimal conditions was 600 times greater than the amount of bromine released in CHBr_3 (Table 2). The amount of HOBr released in low light without added H_2O_2 was nearly 100 times greater than the amount of bromine released in CHBr_3 (using results in Table 1).

The reaction of HOBr and DOM is very rapid, with a half-life of $<7 \text{ ms}$ in high-DOM-containing surface waters ($400 \text{ } \mu\text{mol C L}^{-1}$; Jaworski and Helz 1985). In addition to producing polybromomethanes, diatom-released HOBr will react with reactive components of DOM to form nonvolatile brominated organics, such as haloacetic acids and haloacetonitrils, as they are in water disinfection procedures (Krasner et al. 1996). Most likely, the amount of polybromomethanes produced is a fraction of the total HOBr released; the amount produced will depend upon the quantity and quality of DOM present.

The release of HOBr and HOI by diatoms (and perhaps other phytoplankton) may be important in polybromo- and polyiodo-methane production in polar and tropical marine environments. Cota and Sturges (1997) estimated emissions from ice algae at $124\text{--}5434 \text{ ng CHBr}_3 \text{ g dry weight (wt)}^{-1} \text{ h}^{-1}$ (mean $998 \text{ ng CHBr}_3 \text{ g dry wt}^{-1} \text{ h}^{-1}$). Using a mean value for diatom cell biomass of $0.023 \text{ } \mu\text{g dry wt}^{-1} \text{ cell}^{-1}$ (Parsons et al. 1961; not counting the extremely large species *Coscinodiscus*) gave a bromine release rate of $0.03\text{--}1.5 \text{ fmol Br cell}^{-1} \text{ h}^{-1}$ (mean $0.3 \text{ fmol Br cell}^{-1} \text{ h}^{-1}$). The release rate of bromine in CHBr_3 is 230 times lower than the mean release of bromine as HOBr for the three polar species in Table 2 ($69 \text{ fmol HOBr cell}^{-1} \text{ h}^{-1}$). If CHBr_3 is formed from the released HOBr reacting with DOM, assuming equality in HOBr release from sea-ice algae and our cultures, between 27% and 99% of HOBr released is not accounted for in the emitted CHBr_3 .

The reported HOBr release rates are based on optimum incubation conditions, including that for H_2O_2 ; however, the rates measured from our incubation studies in the light may approximate such rates in natural algal assemblages. With no added H_2O_2 to the incubation of *P. galcials* in the light, HOBr release was still large, at 24% the bromination rate, in the light with optimal H_2O_2 concentration (Table 1). The amount of light used during the incubations was $0.6 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, which is much less than that found in sea-ice habitats during the spring and summer ($1\text{--}5 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ under ice and $12\text{--}25 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on the ice surface in spring; Smith et al. 1989). A higher photon flux density (PFD) will result in greater H_2O_2 production from photosynthesis. An increase in the external bromination of PR has been shown with seaweeds exposed to greater PFD (Wever et al. 1991). The resulting concentration of H_2O_2 in the diatom apoplast will be higher than in the surrounding seawater.

Assuming that the sea-ice algal assemblage release HOBr at similar rates to those of the polar diatoms that we measured, and assuming an ice algal total Chl concentration of 22 mg Chl m^{-2} (Sturges et al. 1997); using the total Chl-normalized values in Table 2, we estimated that sea-ice

algae could release up to $0.9\text{--}40 \text{ } \mu\text{mol HOBr h}^{-1} \text{ m}^{-2}$. This range may be underestimated because of higher Chl values of our cultures as compared to the natural population. The HOBr and HOI release rates normalized to total Chl (Table 2) are most likely much lower than those found in nature, because the Chl values were enhanced by high available nitrogen in the culture medium.

Much of the released reactive bromine and iodine ($\text{Br}_2 + \text{HOBr}$, and $\text{I}_2 + \text{HOI}$, respectively) probably reacts with the surrounding DOM. Sea-ice assemblages in bottom ice contain high levels of DOM, $300\text{--}3000 \text{ } \mu\text{mol C L}^{-1}$, although when the seasonal ice melts, the released diatoms would be surrounded by much lower DOM concentrations (Smith et al. 1997). Algal-released HOBr may also react with bromide or chloride to form Br_2 and BrCl in interstitial hypersaline brine channels of both permanent and seasonal sea ice. The amount of HOBr in sea ice would not only be dependent upon its release rate from sea-ice algae and the amount of DOM present but would also be dependent upon the equilibrium with molecular bromine ($\text{Br}_2 + \text{H}_2\text{O} \leftrightarrow \text{HOBr} + \text{Br}^- + \text{H}^+$; bromine hydrolysis reaction). The acidic apoplast should favor Br_2 formation, while seawater pH should promote Br_2 hydrolysis. The kinetics of Br_2 formation are rapid, and equilibrium constants have been empirically determined (Beckwith et al. 1996). Using these results, the equilibrium constant for bromine hydrolysis in seawater (ionic strength = 0.7 mol L^{-1} , temp = 6.3°C) was calculated ($K = 1 \times 10^{-9} [\text{mol L}^{-1}]^2$) to estimate the concentration of Br_2 in sea ice and melt water. The sea-ice concentration of HOBr was derived from the areal production rate ($0.9\text{--}40 \text{ } \mu\text{mol h}^{-1} \text{ m}^{-2}$), as described in the preceding paragraph, and assuming ice algae cover of $\frac{1}{2} \text{ m}$ in depth (Sturges et al. 1997) with no HOBr loss over a 1-h period. The calculated HOBr concentration after 1 h would be $1.8\text{--}79 \text{ nmol L}^{-1}$. Using these values and seawater of pH = 8 and $[\text{Br}^-] = 840 \text{ } \mu\text{mol L}^{-1}$, we calculate the potential Br_2 concentration in sea ice to measure between 14 and 650 pmol L^{-1} , approximately 0.8% of HOBr present. Volatile Br_2 and BrCl could possibly find their way to the marine boundary layer from channels formed from ice fractures or seasonal ice melts. When seasonal ice melts, released sea-ice algae are found in the melt water near the surface, where they show subsequent growth (Carpenter et al. 2007). This might also lead to the transport of these halogens into the troposphere. Assuming sea-ice algae are uniformly present within the entire Arctic ice field, the area-normalized estimated rate of Br_2 production is $0.007\text{--}0.32 \text{ } \mu\text{mol h}^{-1} \text{ m}^{-2}$ ($0.014\text{--}0.64 \text{ } \mu\text{mol Br h}^{-1} \text{ m}^{-2}$). This value is close to the emission rate of $0.02 \text{ } \mu\text{mol Br m}^{-2} \text{ h}^{-1}$ needed to account for the buildup of bromine in the atmosphere during the Arctic spring, which has been primarily attributed to nonbiological processes (Martinez et al. 1999). If 99% of the HOBr released reacts with DOM, the remaining 1% could still produce a significant amount of Br_2 ($0.07\text{--}3.2 \text{ nmol m}^{-2} \text{ h}^{-1}$). We raise the possibility that sea-ice algae may be a source of Br_2 to the polar troposphere. Further work and analysis needs to be done to test this hypotheses.

Sea-ice algae are a significant source of iodine in the Arctic and Antarctic boundary layer via their production of

the photoreactive CH_2I_2 (Carpenter et al. 2007; Saiz-Lopez et al. 2007). A mechanism producing CH_2I_2 from a reaction between DOM and abiotically produced reactive iodine has been proposed (Carpenter et al. 2005). Our results indicate that diiodomethane may be produced from a reaction of DOM with HOI released from sea-ice diatoms inhabiting the melt water. This mechanism supports the hypothesis of in situ CH_2I_2 production in surface melt water (Carpenter et al. 2007). Calculation of the release rate of HOI by sea-ice algae, similar to that obtained for HOBr above (optimal conditions), yields a range of 0.4–60 $\mu\text{mol HOI h}^{-1} \text{m}^{-2}$.

Bromoform and CH_2Br_2 are generally supersaturated in tropical oceanic seawater (Butler et al. 2007). These waters are a significant source of CHBr_3 , independent from coastal production; a subsurface chlorophyll maximum associated with the tropical thermocline is a CHBr_3 source (Quack et al. 2004). The warm-water species *T. oceanica* did show significant release of HOBr, while the other species *F. pinnata* showed no activity (Table 2). Clearly more warm-water and tropical species need to be examined.

A lack of strong correlation between CHBr_3 production and diatom presence was observed in the Mauritanian upwelling (Quack et al. 2007). If CHBr_3 production occurs primarily from the reaction of released reactive bromine by diatoms and DOM, the process may have been DOM limited. Quack et al. (2007) have speculated that 'free peroxidase' may contribute to the abiotic CHBr_3 production in seawater. It is possible that the extracellular BrPO of diatoms may still be active even after cell death.

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References

- ALMEIDA, M., AND OTHERS. 2001. Vanadium haloperoxidases from brown algae of the Laminariaceae family. *Phytochemistry* **57**: 633–642.
- ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1–15.
- BECKWITH, R. C., T. X. WANG, AND D. W. MARGERUM. 1996. Equilibrium and kinetics of bromine hydrolysis. *Inorg. Chem.* **35**: 995–1000.
- BRISKIN, P. D. 1990. The plasma membrane H^+ -ATPase of higher plant cells: Biochemistry and transport function. *Biochim. Biophys. Acta* **1019**: 95–109.
- BUTLER, J. H., AND OTHERS. 2007. Oceanic distributions and emissions of short-lived halocarbons. *Glob. Biogeochem. Cycles* **21**: GB1023, doi:10.1029/2006GB002732.
- CARPENTER, L. J. 2003. Iodine in the marine boundary layer. *Chem. Rev.* **103**: 4953–4962.
- , D. J. WEVILL, C. J. PALMER, AND J. MICHELS. 2007. Depth profiles of volatile iodine and bromine-containing halocarbons in coastal Antarctic waters. *Mar. Chem.* **103**: 227–236.
- , AND OTHERS. 2005. Abiotic source of reactive organic halogens in the sub-arctic atmosphere? *Environ. Sci. Technol.* **39**: 8812–8816.
- CLASS, T., AND K. BALLSCHMITER. 1988. Chemistry of organic traces in air, VIII, sources and distribution of bromo- and bromochloromethanes in marine air and surface water of the Atlantic Ocean. *J. Atmos. Chem.* **6**: 35–46.
- COTA, G. F., AND W. T. STURGES. 1997. Biogenic bromine production in the Arctic. *Mar. Chem.* **56**: 181–192.
- DE LA CUESTA, J. L. 1996. The role of marine phytoplankton in the biogeochemical cycling of iodine. M.S. thesis. California State Univ.–Long Beach.
- EVERETT, R. R., H. S. SOEDJAK, AND A. BUTLER. 1990. Mechanism of dioxygen formation catalyzed by vanadium bromoperoxidase. *J. Biol. Chem.* **265**: 15671–15679.
- FORTI, G., AND P. GEROLA. 1977. Inhibition of photosynthesis by azide and cyanide and the role of oxygen in photosynthesis. *Plant Physiol.* **59**: 859–862.
- FOSTER, K. L., R. A. PLASTRIDGE, J. W. BOTTENHEIM, P. B. SHEPSON, B. J. FINLAYSON-PITTS, AND C. W. SPICER. 2001. The role of Br_2 and BrCl in surface ozone destruction at polar sunrise. *Science* **291**: 471–474.
- HILL, V. L. 2008. External bromoperoxidases in marine diatoms. M.S. thesis. California State Univ.–Long Beach.
- HOBBIE, J. E., R. J. DALEY, AND S. JASPER. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225–1228.
- HOSHAW, R. W., AND J. R. ROSOWSKI. 1973. Methods for microscopic algae, p. 53–67. *In* J. R. Stein [ed.], *Handbook of phycological methods, culture methods and growth measurements*. Cambridge Univ. Press.
- JAWORSKI, D. A., AND G. R. HELZ. 1985. Rapid consumption of bromine oxidants in river and estuarine waters. *Environ. Sci. Technol.* **19**: 1188–1191.
- JOHNSON, L. M., K. D. HOAGLAND, AND M. R. GRETZ. 1995. Effects of bromide and iodide on stalk secretion in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). *J. Phycol.* **31**: 401–412.
- KALESCHKE, L., AND OTHERS. 2004. Frost flowers on sea ice as a source of sea salt and their influence on tropospheric halogen chemistry. *Geophys. Res. Lett.* **31**: L16114, doi:10.1029/2004GL020655.
- KRASNER, S. W., M. J. SCLIMENTI, R. CHINN, Z. K. CHOWDHURY, AND D. M. OWEN. 1996. The impact of TOC and bromide on chlorination by-product formation, p. 59–90. *In* R. A. Minear and G. L. Amy [eds.], *Disinfection by-products in water treatment*. CCR Press.
- KRENN, B. E., M. G. TROMP, AND R. WEVER. 1989. The brown alga *Ascophyllum nodosum* contains two different vanadium bromoperoxidases. *J. Biol. Chem.* **264**: 19287–19292.
- KUPPER, F. C., D. G. MULLER, A. F. PETERS, B. KLOAREG, AND P. POTIN. 2002. Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of sporophytes of laminariales. *J. Chem. Ecol.* **28**: 2057–2081.
- , N. SCHWEIGERT, E. AR GALL, J. M. LEGENDRE, H. VILTER, AND B. KLOAREG. 1998. Iodine uptake in laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* **207**: 163–171.
- LUTHER, G. W., C. B. SWARTZ, AND W. J. ULLMAN. 1988. Direct determination of iodide in seawater by cathodic stripping square wave voltammetry. *Anal. Chem.* **60**: 1721–1724.
- MANLEY, S. L., AND P. E. BARBERO. 2001. Physiological constraints on bromoform (CHBr_3) production by *Ulva lactuca* (chlorophyta). *Limnol. Oceanogr.* **46**: 1392–1399.

- MARTINEZ, M., T. ARNOLD, AND D. PERNER. 1999. The role of bromine and chlorine chemistry for arctic ozone depletion events in ny-ålesund and comparison with model calculations. *Ann. Geophys.* **17**: 941–956.
- MILLER, W. L., AND D. R. KESTER. 1994. Peroxide variations in the Sargasso Sea. *Mar. Chem.* **48**: 17–29.
- MOORE, R. M., M. WEBB, R. TOKARCZYK, AND R. WEVER. 1996. Bromoperoxidase and iodoperoxidase enzymes and production of halogenated methanes in marine diatom cultures. *J. Geophys. Res.* **101**: 20899–20908.
- MURPHY, C., R. MOORE, AND R. WHITE. 2000. Peroxidases from marine microalgae. *J. Appl. Phycol.* **12**: 507–513.
- PARSONS, T. R., K. STEPHENS, AND J. D. H. STRICKLAND. 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Board Can.* **18**: 1001–1016.
- QUACK, B., E. ATLAS, G. PETRICK, V. STROUD, S. SCHAUFFLER, AND D. W. R. WALLACE. 2004. Oceanic bromoform sources for the tropical atmosphere. *Geophys. Res. Lett.* **31**: L23S05, doi:10.1029/2004GL020597.
- , I. PEEKEN, G. PETRICK, AND K. NACHTIGALL. 2007. Oceanic distributions and sources of bromoform and dibromomethane in Mauritanian upwelling. *J. Geophys. Res.* **112**: C10006, doi:10.1029/2006JC003803.
- , AND D. W. R. WALLACE. 2003. Air-sea flux of bromoform: Controls, rates, and implications. *Glob. Biogeochem. Cycles* **17**: 1023, doi:10.1029/2002GB001890.
- SAIZ-LOPEZ, A., A. S. MAHAJAN, R. A. SALMON, S. J. B. BAUGUITTE, A. E. JONES, H. K. ROSCOE, AND J. M. C. PLANE. 2007. Boundary layer halogens in coastal Antarctica. *Science* **317**: 348–351.
- SMITH, R. E. H., P. CLEMENT, AND E. J. HEAD. 1989. Biosynthesis and photosynthate allocation patterns of arctic ice algae. *Limnol. Oceanogr.* **34**: 591–605.
- , M. GOSSELIN, S. KUDOH, B. ROBINEAU, AND S. TAGUCHI. 1997. DOC and its relationship to algae in bottom ice communities. *J. Mar. Sys.* **11**: 71–80.
- SOEDJAK, H. S., J. V. WALKER, AND A. BUTLER. 1995. Inhibition and inactivation of vanadium bromoperoxidase by the substrate hydrogen peroxide and further mechanistic studies. *Biochemistry* **34**: 12689–12696.
- STURGES, W. T., G. F. COTA, AND P. T. BUCKLEY. 1992. Bromoform emission from arctic ice algae. *Nature* **358**: 660–662.
- , AND ———. 1997. Vertical profiles of bromoform in snow, sea ice, and seawater in the Canadian arctic. *J. Geophys. Res.* **102**: 25073–25083.
- SUN, J., AND D. LIU. 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. *J. Plankton Res.* **25**: 1331–1346.
- TSCHIRRET-GUTH, R., AND A. BUTLER. 1994. Evidence for organic substrate binding to vanadium bromoperoxidase. *J. Am. Chem. Soc.* **116**: 411–412.
- TWINER, M. J., AND C. G. TRICK. 2000. Possible physiological mechanisms for the production of hydrogen peroxide by the ichthyotoxic flagellate *Heterosigma akashiwo*. *J. Plankton Res.* **22**: 1961–1975.
- VAN PEE, K. H., AND F. LINGENS. 1985. Purification of bromoperoxidase from *Pseudomonas aureofaciens*. *J. Bacteriol.* **161**: 1171–1175.
- VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Yale Univ. Press.
- VREELAND, V., AND L. EPSTEIN. 1996. Analysis of plant-substratum adhesives, p. 95–116. *In* H. F. Linskins and J. F. Jackson [eds.], *Plant cell wall analysis: Modern methods of plant analysis*. Springer.
- WEVER, R., M. G. M. TROMP, B. E. KRENN, A. MARJANI, AND M. VAN TOL. 1991. Brominating activity of the seaweed *Ascophyllum nodosum*: Impact on the biosphere. *Environ. Sci. Technol.* **25**: 446–449.

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