



#873 Bromoperoxidase Catalyzed Production of Polybromomethanes from Seawater DOM



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Abstract

Bromoform and other bromomethanes were produced from coastal seawater upon the addition of algal bromoperoxidase (BrPO; 0.002 units per ml) and hydrogen peroxide (23 μM). Their production utilizes a component of DOM, which is most likely to be the highly reactive "biologically labile" fraction. Production ranged from 17 to 110 fmol CHBr₃ · L⁻¹ · h⁻¹ (mean 46), and 54 to 350 fmol organo-Br · L⁻¹ · h⁻¹ (mean 147) from polybromomethanes. Fractionation of DOM by ultrafiltration demonstrated bromoform production occurred at similar rates in three MW fractions between 1,000 and 100,000 Daltons and at lower rates in the low MW fraction (<1,000) and high MW fraction (>100,000). Diatoms contain up to 6 × 10⁻⁶ units per cell of extracellular BrPO which corresponds to 130 BrPO units per mg Chl a. We estimate that a moderate diatom bloom (50 mg Chl a · m⁻²) could produce up to 10 pmole of bromoform · m⁻² · d⁻¹ from BrPO catalyzed bromination of DOM, assuming a high hydrogen peroxide concentrations could be maintained in the periplast (region between the frustule and cell membrane) to ensure high BrPO activity.

Introduction

Polybromomethanes:

- supply organo-bromine and iodine to the atmosphere.
 - promote stratospheric ozone destruction (Sturges *et al.*, 2000).
 - bromomethanes partially responsible for tropospheric ozone decline during Arctic spring.
- include bromoform (CHBr₃) the dominant polybromomethane (Quack & Wallace, 2003).
- are produced by marine algae (seaweeds & phytoplankton) via bromoperoxidase (Moore *et al.*, 1996).
 - seaweeds produce ~70% of the global CHBr₃.
 - sea ice phytoplankton produce significant amounts of polar CHBr₃ (Sturges *et al.*, 2000).

Extracellular bromoperoxidase (BrPO):

- found between the cell membrane and cell wall (apoplast) of seaweeds and diatoms (Wever *et al.*, 1991; Manley & Hill, 2006)
- oxidize bromide and iodide with H₂O₂ to HOBr and HOI which are released from the active site of BrPO.

Dissolved Organic Carbon (DOC):

- in oceans comes primarily from cell lysis, exudation and excretion.
 - related to oceanic and coastal primary production; terrestrial input is significant in certain coastal regions.
 - contain labile and semilabile components which are biologically reactive and biologically recalcitrant components.
- in drinking water forms trihalomethanes (THMS) during the disinfection treatment with Cl₂, Br₂ or ozone (Skechell *et al.*, 1995).
 - CHBr₃, the common by-product.
 - common precursors to THMS include phenolic compounds (lignin derived), β-diketones and β-keto acids.
 - Brominated by algal BrPO has been suggested to produce polybromo- methanes (Wever *et al.*, 1991)
 - half the CHBr₃ produced by the seaweed *Ulva* attributed to the BrPO catalyzed bromination of DOC (Manley and Barbero, 1999)

Hypotheses

- Polybromomethanes are produced from the reaction of the reactive DOC components with HOBr released from extracellular BrPO.
- The abundance of the reactive DOC components that form polybromomethanes varies seasonally and spatially due to differences in coastal production, terrestrial input an *in situ* transformations.
- Certain molecular weight (MW) fractions of seawater DOC are more reactive in forming polyhalomethanes than others.

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Materials and Methods

Production of polybromomethanes from the addition of BrPO:

Seawater samples were collected from the end of the Seal Beach pier, CA. and 2 km off-shore (Fig. 1) and prefiltered (0.22 μm dia.) at low vacuum; Salinity, temperature and phytoplankton I.D. also determined (data not shown). Standard reaction: 20 μl of BrPO stock solution (0.002 units · ml⁻¹), 200 μl of 0.22 M Br⁻, 50 μl of H₂O₂ stock (23 μM final conc.) added to 1 ml of prefiltered seawater; vial sealed and incubated at 25°C for 2.5 hrs. Reaction stopped (BrPO denatured) in 60°C water bath for 10 min. Analysis: vials cooled to 25°C, 2ml headspace analyzed by GC-ECD. Total amount of each compound calculated using Henry's constants (Moore *et al.*, 1995); bromomethanes production rates of from the experimental group were compared with control (no BrPO). The potential inhibition of BrPO activity by seawater was concurrently assayed using phenol red as the substrate.

To determine the reactivity of ultrafractionated DOC fractions:

Ultrafiltration (UHP N₂ at 4°C; filters prewashed with double DI H₂O and 35% NaCl) fractionated and concentrated (3-fold) DOC into five MW size classes: 0.2 μm-100,000 Da, 100,000-30,000 Da, 30,000-10,000 Da, 10,000-1,000 Da and <1,000 Da. Polybromomethane production by each fraction was determine as previously described.

Results

Seal Beach Pier

- CHBr₃ production rate was significantly higher than all other bromomethanes and the control (no BrPO) during the six-month sampling period (Fig. 2a).
- Total organo-Br production was significantly higher than the controls except in June.
- During the first month of the wet season (December), the production rate of CHBr₃ was approximately five-fold higher than that in dry season (July).
- Seasonal differences were not based on the presence of a BrPO inhibitor in seawater (data not shown).

Seal Beach Pier vs. Offshore

- In July, August and December, the production rates of all polybromomethanes (except CH₂Br₂) and total organo-Br from seawater sampled at pier were significantly higher than those sampled offshore (Fig. 2B).

Ultrafiltration of seawater DOC

- The DOC size classes of 0.2 μm (~ 500,000 Da) -100,000 Da and <1,000 Da. showed lower production of CHBr₃ and total organo-Br production rates than all other size classes (Fig. 3).
- Production of chloro- substituted bromomethanes and CH₂Br₂ lower than CHBr₃ and total organo-Br.

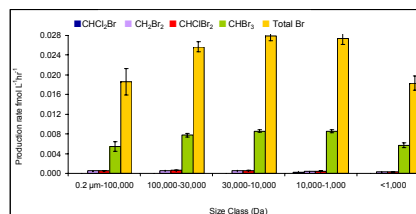


Figure 3. Polybromomethane production from size fractionated DOC (bar = range; n=2).

Conclusions

- Components of seawater DOC produce polybromomethanes in the presence of BrPO and Br⁻ and H₂O₂; bromoform is the major product.** Extracellular BrPO of phytoplankton and seaweeds may similarly release HOBr which reacts with DOC to produce CHBr₃ & CH₂Br₂; the chlorobromomethanes being produced by chloride substitution (Class and Ballschmiter, 1988). High concentrations of H₂O₂ can be maintained in the extracellular space of algae to facilitate high BrPO activity.
- Increased DOC derived polybromomethanes occurred during the rainy month of December presumably due to increased concentration of DOC from coastal runoff.** This effect was much less evident in offshore seawater.
- Polybromomethane production occurred in all DOC size classes.** DOC can contain humic substances (5-25% of DOC; Benner, 2002), and other compounds that could be halogenated to eventually form polyhalomethanes. Such reactive compounds evidently are not confined to a single MW size class.
- We estimate that a moderate diatom bloom could produce up to 14 pmoles CHBr₃ and 90 pmoles of organo-Br · m⁻² · d⁻¹ from DOC.** This is based on a maximum number of BrPO units per diatom cell of 6.2 × 10⁴, mg Chl a per cell of 4.74 × 10⁻⁸ (Manley and Hill, 2006), a seawater [H₂O₂] of 70 nM, and a mean production of 46 fmol CHBr₃ L⁻¹ hr⁻¹ and 150 fmol organo-Br L⁻¹ hr⁻¹ (Fig. 1a). Seawater [H₂O₂] ranges from 10 to 1,000 nM, the higher range occurs in the presence of phytoplankton blooms, and the diatom apoplast may maintain a high [H₂O₂]. Therefore, the rate of CHBr₃ production from DOC could be significantly higher. Diatoms can release to 3.8 μmol HOBr · mg Chl a⁻¹ · hr⁻¹ from their external BrPO (Manley and Hill, 2006).

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Figure 1. Sampling locations.

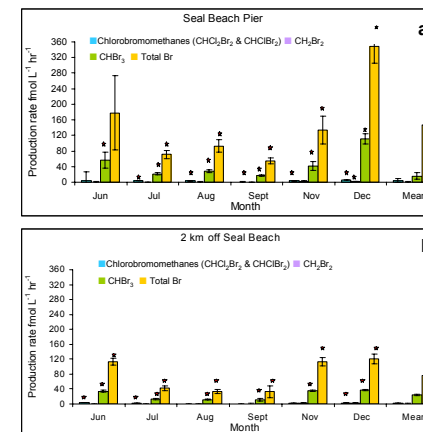


Figure 2. Polybromomethane production rates from coastal (a) & offshore (b) seawater (bar = ± sd; n=3). Red stars indicate the production rate is significantly higher than the control (no BrPO).