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Viewpoint

Biosynthesis of hydroxylated polybrominated diphenyl ethers and the correlation with photosynthetic pigments in the red alga *Ceramium tenuicorne*

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ABSTRACT

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been identified in a variety of marine organisms from different trophic levels indicating a large spread in the environment. There is much evidence pointing towards natural production as the major source of these compounds in nature. However, much is still not known about the natural production of these compounds. Seasonal trend studies have shown large fluctuations in the levels of OH-PBDEs in *Ceramium tenuicorne* from the Baltic Sea. Yet, even though indications of stimuli that can induce the production of these compounds have been observed, none, neither internal nor external, has been assigned to be responsible for the recorded fluctuations. In the present study the possible relationship between the concentration of pigments and that of OH-PBDEs in *C. tenuicorne* has been addressed. Significant correlations were revealed between the concentrations of all OH-PBDEs quantified and the concentrations of both chlorophyll *a* and Σxanthophylls + carotenoids. All of which displayed a concentration peak in mid-July. The levels of OH-PBDEs may be linked to photosynthetic activity, and hence indirectly to photosynthetic pigments, via bromoperoxidase working as a scavenger for hydrogen peroxide formed during photosynthesis. Yet the large apparent investment in producing specific OH-PBDE congeners point towards an targeted production, with a more specific function than being a waste product of photosynthesis. The OH-PBDE congener pattern observed in this study is not agreeable with some currently accepted models for the biosynthesis of these compounds, and indicates a more selective route than previously considered in *C. tenuicorne*.

1. Introduction

Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) are ubiquitous in aquatic environments around the world. OH-PBDEs have for example been detected in biological samples from the great lakes basin of North America (Fernie and Letcher, 2010), the Pacific Ocean (Nomiyama et al., 2011), the Indian Ocean (Gribble, 2010), and the Atlantic Ocean (Strid et al., 2010). In the Baltic Sea the OH-PBDEs are equally ubiquitous throughout the food web, and have been identified in for example mussels (Löfstrand, 2011), fish (Löfstrand, 2011), seals (Routti et al., 2009), and predatory birds (Nordlöf, 2012). Some congeners have even increased significantly in concentration over the last 30 years, as observed in the livers from Baltic herring (Faxneld et al., 2014). This is of great concern due to the number of adverse effects that have been associated with OH-PBDEs, such as disruption of the oxidative phosphorylation (van Boxtel et al., 2008), genotoxicity (Ji et al., 2011), neurotoxicity (Dingemans et al., 2008), and hormonal disturbances (Meerts et al., 2001).

Although OH-PBDEs can be formed by metabolic transformation of anthropogenic PBDEs, used as flame retardants (Hakk and Letcher, 2003), much evidence suggests natural production as the major source of these compounds in the Baltic Sea. Primary producers, such as algae, are known to synthesize brominated organic compounds (BOCs) like bromophenols (BPs) (Dahlgren et al., 2015; Flodin et al., 1999; Gribble, 2010). OH-PBDEs have been detected in a variety of alga species in the Baltic Sea (Löfstrand, 2011). However, contributions to the detected OH-PBDEs may also come from production in the epiphytical community on the surface of the algae

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(Unson et al., 1994). Recently, the production of 2,4,6-tribromophenols (2,4,6-TBP) in isolated clonal material of *Ceramium tenuicorne* (Kützing) Waern (family *Ceramiaceae*) grown in a controlled laboratory setting, confirmed the hypothesis that this specie is a producer of brominated phenolic compounds (Dahlgren et al., 2015).

Production of BOCs by algae is catalyzed by bromoperoxidases (BPOs) (Butler and Walker, 1993). BPOs are antioxidant enzymes that are abundant in marine organisms, and that are present in for example red, brown, and green algae (Butler and Walker, 1993). In plants and algae the processes of photosynthesis and respiration is linked to cellular production of reactive oxygen species (ROS) (Baroli and Niyogi, 2000; Veljovic-Jovanovic, 1998). Hydrogen peroxide (H_2O_2) formed as a result of photosynthetic and respiratory electron transport has been shown to be available as a substrate for BPO (Manley and Barbero, 2001). BPO uses H_2O_2 to oxidize bromide to e.g. hypobromous acid (HOBr), which is sequentially used to brominate organic substrates (Butler and Walker, 1993). The formation of BOCs by BPO may hence provide means of scavenging H_2O_2 before it reaches toxic concentrations (Pedersen et al., 1996). Besides haloperoxidases, several antioxidant systems have evolved to scavenge ROS, and to counteract ROS induced autotoxicity. These includes antioxidant molecules such as carotenoids, and antioxidant enzymes like superoxide dismutase (SOD) and ascorbate peroxidase (Baroli and Niyogi, 2000; Dummermuth et al., 2003; Veljovic-Jovanovic, 1998).

BPO isolated from red algae have been shown to catalyze the bromination of phenol to 2,4,6-TBP *in vitro* (Shang et al., 1994). OH-PBDEs may in turn be formed by dimerization of BPs. The dimerization can also be catalyzed by BPO as previously demonstrated *in vitro* (Lin et al., 2014). The dimerization proceeds via oxidation of BPs to bromophenoxy radicals (Lin et al., 2014; Neilson, 2003), which subsequently undergo biradical coupling (Lin et al., 2014; Neilson, 2003). OH-PBDEs may also be formed in a reaction between a bromophenoxy radical and a BP through loss of a bromine from the latter (Lin et al., 2014).

Stressors such as exposure to pathogens and physical damage can result in a burst of ROS in algae (Collen and Pedersen, 1994; Kupper et al., 2001). Increased production of BOCs algae have in turn been associated with stress induced by ultra-violet (UV) exposure (Laturnus et al., 2004), temperature changes (Abrahamsson et al., 2003), high light intensity (Mtolera et al., 1996), as well as changed salinity and grazing (Dahlgren et al., 2015). As well as being a sink for hydrogen peroxide, the production of halometabolites is believed to contribute to enhanced survival and fitness of the alga through qualities such as antibiotic activities (Butler and Walker, 1993; McConnell and Fenical, 1980). The products of BPO activity in macroalgae have also been indicated as part of defense mechanisms against epiphyte settlement (Ohsawa et al., 2001) as well an anti-feeding deterrent (McConnell and Fenical, 1980). However, the exact role of OH-PBDEs in algal survival is still to be determined.

This study was undertaken in response to an observation made during a previous investigation regarding the variation of OH-PBDEs in *C. tenuicorne* over the summer season. During that investigation the alga samples were noted to possess a variation in their coloration, and seemingly darkened around the same time as the OH-PBDE concentrations peaked within the algae (Dahlgren et al., 2015). The aim of the present study was thusly to investigate the possible correlation between the concentrations of OH-PBDEs and pigments in *C. tenuicorne* over the summer season. Ultimately to increase our knowledge on how and why these compounds are produced by the alga.

The filamentous red macroalga, *C. tenuicorne,* is a common algae in the Baltic Sea. It grows as an epiphyte on other macroalgae, such as *Fucus vesiculosus*, on rocks, or in loose lying algal mats (Bergström and Bergström, 1999), and are present from the surface down to below 10 m. *C. tenuicorne* is an opportunistic fast growing species that may benefit from the nutrient enrichment of the Baltic Sea (Schramm, 1996). This makes this species interesting in the context of OH-PBDEs in the Baltic Sea and their potential increase over time. The major endogenous xanthophylls, carotenoids, and chlorophylls of *C. tenuicorne* include *β*,*β*-carotene, lutein, zeaxanthin and chlorophyll *a* (Chl *a*). Chl *b* is not present in *C. tenuicorne* (Bianchi et al., 1997). *C. tenuicorne* from the northern Baltic Sea has also been shown to contain high levels of phycobiliprotein pigments, with phycoerythtrin being the most abundant (Bäck and Likolammi, 2004).

2. Results

2.1. Concentrations and correlations of pigments and OH-PBDEs/BPs

Spectrophotometric analyses showed that the concentration of Σxanthophylls + carotenoids (Σx + c) in general was four times lower than that of Chl *a* in the algae over the summer season (see Table 1). The levels of Σx + c also fluctuated in accordance with that of Chl *a*, with a clear statistical correlation between the concentrations of the two (see Fig. 1, data from the linear regression analysis is presented in the supplementary data (SD), Table S1). The concentrations of all seven OH-PBDE congeners quantified in this study, individually and as a sum (ΣOH-PBDE), showed statistical correlations with both the Σx + c concentration and the Chl *a* concentration (with 95% confidence intervals). Overall the OH-PBDE trends were better correlated with the Σx + c trend than with the Chl *a* trend (SD, Table S1), and better correlated on wet weight basis than dry weight, while still significant on both (SD, Table S1). 2,4-dibromophenol (2,4-DBP) was also correlated with the pigment concentrations, while 2,4,6-TBP was not. Both the pigments and the OH-PBDEs showed two concentration peaks, one smaller on the 19th of June and one larger on the 19th of July (see Fig. 1). The two peaks were more pronounced for the general OH-PBDE trend (depicted by the ΣOH-PBDE concentration trend in Fig. 1) than for the Σx + c and Chl *a* trends.

The congener pattern was relatively stable over the studied period and was dominated by 6-OH-BDE85 followed by 6-OH-BDE137 (Table 1). The quantified seven OH-PBDEs included all (5) dominant peaks within the region of BP dimerization products containing 3–6 bromines in the gas chromatography mass spectrometry (GC-MS) chromatogram, and two minor peaks (see Fig. 2). The two quantified BPs were utterly dominant within the BP region throughout the sampling period. In the samples from mid-June another compound group of large brominated weak-acids was also detected, which appeared and then disappeared very rapidly, and seemingly independent of the pigments and OH-PBDEs. These compounds eluted later than the OH-PBDEs on the GC-MS system, and only one was of high enough concentration to produce a proper mass spectrum in full-scan mode. This mass spectrum showed an isotope pattern indicating six bromines in the molecule, but the molecular weight (*m/z* 734, for the methylated derivative) was not consistent with a BP dimer, in this case it weighed too much indicating a more complex backbone structure.

2.2. Correlations with external factors

Acquired data for photosynthetic photon density (PPD) and UV could not be correlated with any measured concentrations of pigments or bromophenolic compounds in the algae (see Fig. 1). Recorded surface temperature at the sampling area increased from

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