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Release of reactive organic halogens by the brown macroalga *Saccharina latissima* after exposure to ultraviolet radiation

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Abstract

The brown macroalga *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (formerly *Laminaria saccharina* [L.] Lamouroux) was exposed to ultraviolet radiation (UVR) in the mW UV-A and mW UV-B range in the laboratory for up to 28 days. The release rates of volatile organohalogens, such as chloroform, bromoform, dibromomethane and methyl iodide, were determined. From these rates, the total emission of reactive organic halogens was calculated. The results revealed that exposure to UVR significantly affected the emission of reactive organic halogens by the macroalga under investigation. An increase in the release of reactive organic iodine was observed for the algal species. In contrast, for reactive organic bromine and reactive organic chlorine, a decrease in emission by the macroalga was observed. Apparently, the potential for increased levels of UVR resulting from further ongoing destruction of the stratospheric ozone layer may increase the importance of marine macroalgae in atmospheric reactions involving organic halogens.

The increase of surface ultraviolet radiation (UVR) caused by the depletion of stratospheric ozone is still a major environmental concern. It is assumed that the level of UVR reaching the Earth's surface will continue to increase until the ozone layer recovers (e.g., Shindell et al. 1998). The ongoing warming of the troposphere further supports atmospheric conditions involved in the destruction of stratospheric ozone over the polar regions (Shindell et al. 1998). It is generally agreed that the depletion of stratospheric ozone is largely caused by the emission of reactive halogens derived from volatile organohalogens of anthropogenic and natural origin. Once in the atmosphere, these organic halogens interfere with and modulate many chemical processes (Daniel et al. 1999; Solomon 1999). Marine macroalgae are an important source of reactive organic halogens, especially in coastal ecosystems (Carpenter & Liss 2000; Laturnus 2001). It is still not known exactly why macroalgae form volatile organic halogens, but it has been suggested that this process may be caused by oxidative stress (Pedersén et al. 1996; Weinberger et al. 2007). As UVR is known to cause stress in biota (Franklin & Forster 1997), it is possible that it could induce the formation of reactive organic halogens by these algae (e.g., Laturnus et al. 2004). Hence, elevated UVR may lead to a feedback loop in which higher emissions of organic halogens by algae lead to a further decrease of the ozone layer. The present study examines whether or not elevated levels of UVR cause an increased emission of reactive organic halogens by macroalgae. The brown algal species *Saccharina latissima* was chosen for its widespread occurrence and high biomass in the Arctic Circle and other cold-temperate environments (Lüning 1990). Furthermore, brown algae are known to be the strongest accumulators of iodine among living organisms, and are considered to be a major pump in the global biogeochemical cycle of iodine (Küpper et al. 2008).

Material and methods

Six-month-old plants of the brown macroalgal species *S. latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders were exposed to combined photosynthetic active radiation (PAR), UV-A and UV-B radiation under controlled laboratory conditions for 28 days. The algae were grown from spores of a sporophyte of *S. latissima*

isolated in Svalbard. The culture was unialgal, but not sterile. PAR was provided by one cool-white fluorescent neon tube (L58/W19, 23 μmol photons m^{-2} s^{-1}, 700–400 nm; Osram, Munich). UV-A (7.7 W m^{-2}, 320–400 nm) and UV-B (0.70 W m^{-2}, 280–320 nm) were provided by two UV-A-340 fluorescence tubes (Q-Panel, Cleveland, OH, USA). The photon flux of PAR was measured with a LI-185 B quantum radiometer and a LI-190 B 2π quantum sensor (LI-COR Biosciences, Lincoln, NE, USA). UV-A and UV-B photon fluxes were measured with a Solarlight PMA 2100 broadband radiometer equipped with a UV-A sensor PMA 2110 and a UV-B sensor PMA 2106 (Solarlight, Philadelphia, PA, USA). Surface UVR levels of around 19 W m^{-2} UV-A and 1.1 W m^{-2} UV-B are common on clear days at noon (Bischof et al. 1998). The UVR applied in the experiment corresponds to 0.63 W m^{-2} weighted irradiance using the action spectrum for general plant damage (Bischof et al. 2000). During the exposure of the macroalgae to UVR, subsamples were taken at days 1, 14 and 28. For the subsamples, entire macroalgal thalli (3–5 g fresh weight for each sample) were incubated in quartz glass flasks (volume 250–300 ml) sealed with glass stoppers and polytetrafluoroethylene (PTFE) collars. The flasks were filled with nutrient-enriched sterile seawater (Provasoli enriched seawater) collected from the North Sea so that no headspace remained (Starr & Zeikus 1993). A 4-h incubation period was chosen to allow for the release of detectable quantities of reactive organic halogens by the macroalgae used in the experiment. The incubation period was chosen to coincide with the middle of the daylight period. During the incubation period, the quartz glass flasks were gently shaken (15 rpm) to support the distribution of the reactive halogen-containing volatile organic compounds in the medium inside the glass flasks. No visible damage of the algal sample tissue was observed for the duration of the experiment. Five replicates of the macroalgal species were performed in each incubation experiment. Control experiments were conducted by exposing the culture medium without algae to the same conditions as the algal cultures.

After termination of the experiment, the culture medium containing the reactive halogen-containing volatile organic compounds was placed in 120-mL glass bottles. The bottles were sealed with crimper caps, that is, aluminium caps with PTFE septa (Chromacol, Welwyn Garden City, Herts, UK), and stored at 4°C until they were analysed. Analysis of the first replicate started immediately after termination of the incubation experiment. Prior to analysis, the sample glass bottles were kept at ca. 20°C and in the dark for 30 min. One hundred millilitres of the culture medium in the sample glass bottle was analysed for volatile organohalogens by automated purge-and-trap gas chromatography and electron capture detection with liquid nitrogen for pre-concentration (CP 9000 instrument with automated purge-and-trap injector; Chrompack, Middelburg, the Netherlands). The sample was transferred with a syringe into the purge-and-trap unit. Helium was used as the purge gas at a purge flow of 40 mL min^{-1} and a purge time of 15 min. The compounds were separated on an Rt_x-volatiles column (40 m length, inner diameter 0.32 mm, film thickness 3 μm; Restek, Bellefonte, PA, USA). The temperature programme was set to 50°C isothermal for 10 min, a heating rate of 4°C min^{-1} and then 150°C isothermal for 5 min. The total run time was 40 min. Identification of the compounds and calculation of the compound concentrations was carried out by adding a calibration standard of the pure compounds (p.a.) in methanol directly to 100 mL pre-purge Milli-Q water. The water containing the standard was then purged for 15 min, and the results for the areas were used to calculate the compound concentrations corrected for recovery. Detection limits ranged from 0.02 to 0.12 pmol L^{-1}, and recovery efficiencies ranged from 47 to 100%. Randomly selected samples were analysed on a column with a different stationary phase (SPB-624 column, length 60 m, inner diameter 0.25 mm, film thickness 1.4 μm; Supelco, Bellefonte, PA, USA). Comparisons of the results from the samples with results from a calibration standard run on the same column were made to increase the reliability of the identified compounds.

Results

In the experiment, the incubation medium used was seawater, which itself contained reactive halogen-containing volatile organic compounds. The concentrations in the seawater medium were within the concentration ranges previously detected in seawater samples (Table 1). The effect of UVR on these volatile organohalogens was investigated prior to the incubation experiment. Except for methyl iodide, the concentrations of volatile organohalogens in the culture medium did not change significantly when exposed to UVR (Table 1). The levels of emitted reactive organic halogens were calculated using the quantities of the individual volatile organohalogens released by the algae. Thus, reactive organic chlorine is the molar sum of chlorine from CH_2Cl_2, $CHCl_3$, 1,1,1-CH_3CCl_3, CCl_4, C_2Cl_4, $CHBrCl_2$, $CHBr_2Cl$ and CH_2ClI. Reactive organic bromine is the molar sum of bromine from CH_2Br_2, $CHBr_3$, 1,2-$EtBr_2$, $CHBrCl_2$ and $CHBr_2Cl$, and reactive organic iodine is the molar sum of iodine from CH_2I_2 and CH_2ClI. Methyl iodide (CH_3I) has not been considered in the molar sum calculations for reactive organic iodine, as significant levels were found in the incubated seawater without algal samples (see Table 1).

Table 1 Influence of ultraviolet radiation (UVR) on reactive halogen-containing volatile organic compounds in filtered seawater used as the medium for the incubation experiment: one set of seawater samples was exposed for 4 h to photosynthetic active radiation (PAR), and one set was exposed for 4 h to PAR + UVR (A + B).

Concentration in oceans[a]		PAR		PAR + UVR		
Range		Average	Min./Max.	Average	Min./Max.	
(pmol l^{-1})	Compound	(pmol l^{-1})		(pmol l^{-1})		*P* value
0.05–18	CH_3I	7.9	7.3/9.7	13.6	12/15.7	0.004
0.4–40	CH_2ClI	0.47	0.37/0.52	0.53	0.31/0.61	0.389
6.2–48	CH_2I_2	1.73	1.43/1.98	1.40	1.18/2.9	0.569
No data	CH_2Cl_2	223	131/407	167	138/220	0.631
9–63	$CHCl_3$	1.8	1.6/2.3	1.9	1.6/2.4	0.315
No data	CH_3CCl_3	2.5	2.2/2.8	2.3	2.2/2.5	0.410
5.4–16	CCl_4	3.1	2.3/3.4	3.0	2.5/3.3	0.416
19[b]	C_2HCl_3	0.8	0.7/1.0	0.8	0.7/0.9	0.734
4.1[b]	C_2Cl_4	1.8	1.5/2.1	1.8	1.5/2.0	0.807
0.3–16	CH_2Br_2	1.3	0.9/2.6	0.9	0.4/1.2	0.085
0.6–2	$CHBrCl_2$	0.1	0.1/0.2	0.2	0.1/0.3	0.789
0.1–5.1	$CHBr_2Cl$	0.42	0.30/0.44	0.46	0.3/0.6	0.541
1.0[b]	1,2-$EtBr_2$	0.27	0.23/0.29	0.31	0.23/0.36	0.466
3–182	$CHBr_3$	4.0	3.5/5.0	4.0	2.8/5.3	0.688

The difference between treatments was tested with a Mann–Whitney *U*-test at 5% significance level ($n = 5$).

[a] Concentration in oceans as reviewed by Laturnus (2003).

[b] No range was given in the literature.

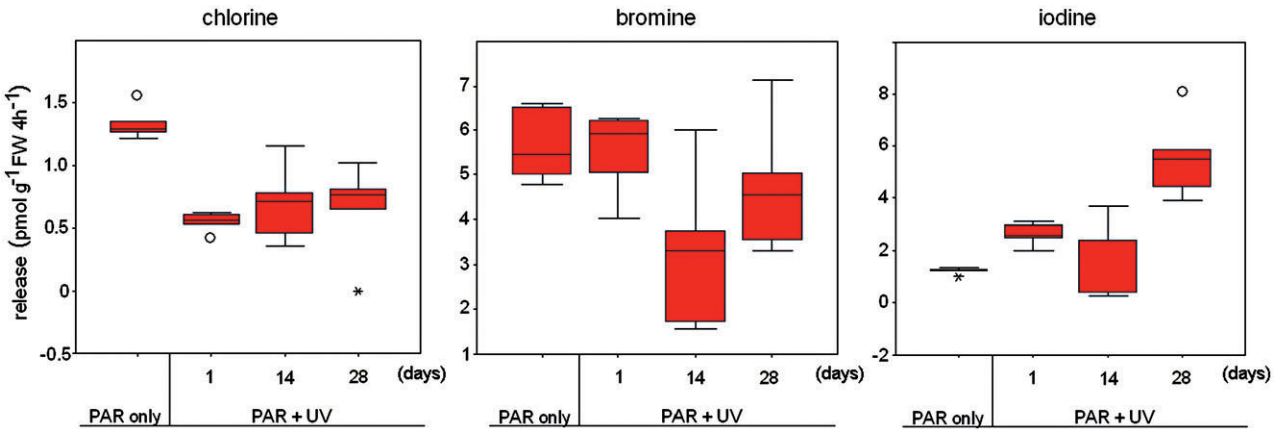

Fig. 1 Emission of reactive organic halogens by the brown macroalga *Saccharina latissima* ex. *Laminaria saccharina* (L.) Lamouroux was exposed for up to 28 days to photosynthetic active radiation (PAR) and ultraviolet radiation (UV-A + UV-B). Results are shown as box plots for five replicates. As the data material was not normally distributed, the central tendency is given as the median, and the variation is presented as the first and third quartiles. The line inside the box represents the median values. The lower and upper edges of the box represent the first and third quartiles, respectively. Minimum and maximum values are given by the error bars of the boxes. Circles represent outliers (values between 1.5 and 3 box lengths from the upper/lower edge of the box). Asterisks indicate extreme values (more than three box lengths from the upper/lower edge of the box.). FW, fresh weight; $n = 5$.

A significant increase in organic iodine emission was observed over the duration of exposure to elevated UVR (Fig. 1; Table 2). In contrast, for bromine, a significant decrease was observed during the first half of the exposure period, and a non-significant increase was observed during the second half of the exposure period (Fig. 1; Table 2). Chlorine exhibited a significant decrease in emission after exposure to UVR, followed by a significant increase with longer durations of UVR exposure (Fig. 1; Table 2). Compared with the emission rates for reactive iodine and bromine, however, chlorine showed sevenfold lower rates.

Table 2. Significant levels for the lease of total halogen by macroalgae exposed to photosynthetic active radiation (PAR) and PAR + ultraviolet radiation (UVR; UV-A + UV-B), as tested by the Mann–Whitney U-test at 5% significance level ($n = 5$)

Mann–Whitney *U*-test	Reactive organic chlorine	Reactive organic bromine	Reactive organic iodine
PAR vs. 1 day UVR	$P = 0.009$	$P = 0.754$	$P = 0.009$
1 day vs. 14 days UVR	$P = 0.465$	$P = 0.047$	$P = 0.175$
14 days vs. 28 days UVR	$P = 0.917$	$P = 0.175$	$P = 0.009$

The release rates are presented in Fig. 1.

Discussion

The results of this study suggest that macroalgae may become more important for regulating the occurrence of reactive organic halogens, provided that UVR levels continue to increase. The biochemical processes underlying the formation of reactive organic halogens are suggested to be based on enzymatically-induced halogenation of organic matter, involving either haloperoxidases or halogenases (Geigert et al. 1984; Butler & Walker 1993; van Pee & Unversucht 2003). In general, halogenation processes and the formation of reactive organic halogens are related to the occurrence of oxidative stress caused by exposure of the algae to hydrogen peroxide or ozone (Palmer et al.2005; Weinberger et al. 2007). Recently, it was suggested that accumulated iodide acts as an inorganic antioxidant, and constitutes an extracellular protection of macroalgae against oxidative stress (Küpper et al. 2008).

In our study, the algae investigated were not subjected to any UVR prior to experimentation, i.e., during cultivation. Exposure to UVR may therefore have triggered additional stress in the algae, which could lead to the formation of additional reactive oxygen species, such as hydrogen peroxide (He & Häder 2002; Dummermuth et al. 2003). For example, in response to such oxidative stress, macroalgae generate and release higher quantities of reactive iodine, which in a second step can react with dissolved organic matter to form volatile organoiodine compounds (McFiggans et al. 2004). Data on possible emissions of inorganic bromine and chlorine are not yet available, but recently, increasing attention has been given to the emission of inorganic iodine by macroalgae (e.g., McFiggans 2005; Palmer et al. 2005; Küpper et al. 2008). The authors described significantly greater levels of inorganic iodine emissions compared with organic iodine under oxidative stress caused by hydrogen peroxide or ozone.

Inorganic iodine emitted into the atmosphere is considered a main source for coastal particles involved in atmospheric radiation and temperature control (McFiggans et al. 2004; McFiggans 2005). A recent field study on the emission of inorganic iodine by *Laminaria digitata* (Hudson) Lamour under elevated levels of ultraviolet radiation (UVR + PAR) showed a more than 14 times higher emission of inorganic iodine, compared with emission under exposure to PAR alone (Grose, Laturnus & Wiencke, unpubl. ms.). The study further revealed a sevenfold lower emission of organic iodine compared with iodide, whereas under exposure to PAR, organic iodine is the dominant species. Thus, exposure of macroalgae to UVR may not only affect the emission of volatile organoiodines but additionally may lead to an increased release of inorganic iodine. Macroalgae of the order Laminariales and the genus *Saccharina* are usually submerged, and are only exposed to the atmosphere during spring tides. A sudden exposure of the macroalgae to UVR would probably lead to a burst of inorganic iodine into the atmosphere. In contrast, macroalgae occurring in the eulitoral are regularly exposed to UVR, and are probably less affected by UVR regarding the emission of iodide. However, data on a wider range of different macroalgae species are still scarce, and more comprehensive evaluations of the atmospheric contribution of inorganic iodine emissions by macroalgae still need to be performed.

During the incubation, and after the experiment was terminated, the algal samples also showed no visible tissue damage, such as colour change or thallus bleaching. However, no specific physiological measurements were made in this regard, so it is not possible here to estimate the stress induced during exposure to UVR. Macroalgae growing directly in the natural habitat may be better adapted to elevated levels of UVR, so their regulation of reactive organic halogens may be enhanced less by UVR than this study suggests.

Despite a worldwide ban on chlorine- and bromine-containing compounds after the discovery of the ozone hole over Antarctica, it remains uncertain when the stratospheric ozone layer will completely recover. Although some studies have reported that regulatory action to reduce the atmospheric input of chlorine- and bromine-containing compounds has already begun to be effective (Pyne 2003; Tabazadeh & Cordero 2004), others have suggested that unknown mechanisms may still be responsible for the loss of stratospheric ozone (Schiermeier 2007). Thus, leaning back and announcing that the ozone problem has now been solved may not be a wise decision at present. Additional threats, such as the

emission of ozone-depleting compounds through uncontrolled and large-scale biomass burning (Manö & Andreae 1994; Yvon-Lewis et al. 2008) and illegal production (Spurgeon 1997) may impede the recovery of the stratospheric ozone layer. Furthermore, greenhouse gases simultaneously cause warming of the troposphere and radiative cooling of the stratosphere (Foster & Shine 1999). Lower stratospheric temperatures promote the formation of polar stratospheric clouds, and support the destruction of stratospheric ozone. Consequently, the levels of UVR reaching the biosphere may still increase in the future, as already intimated earlier (Austin et al. 1992; Shindell et al. 1998). Elevated levels of UVR may increase the environmental input of some reactive organic halogens from marine macroalgae, and perhaps other natural sources known to emit reactive organic halogens. When these halogens are transferred into the atmosphere, they are likely to cause further destruction of stratospheric ozone. Thus, the present picture of the recovery of stratospheric ozone might be even more complex than is widely assumed. Caution might be recommended before giving the ozone layer a clean bill of health and announcing that the threat of stratospheric ozone destruction has been solved.

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