JBIC Journal of Biological Inorganic Chemistry
https://doi.org/10.1007/s00775-018-1539-7

ORIGINAL PAPER

Emission of volatile halogenated compounds, speciation and localization of bromine and iodine in the brown algal genome model *Ectocarpus siliculosus*

Frithjof C. Küpper[1,2] · Eric P. Miller[3] · Stephen J. Andrews[4] · Claire Hughes[5] · Lucy J. Carpenter[4] · Wolfram Meyer-Klaucke[6] · Chiaki Toyama[7] · Yasuyuki Muramatsu[8] · Martin C. Feiters[9] · Carl J. Carrano[3]

Received: 28 November 2017 / Accepted: 28 January 2018

Abstract

This study explores key features of bromine and iodine metabolism in the filamentous brown alga and genomics model *Ectocarpus siliculosus*. Both elements are accumulated in *Ectocarpus*, albeit at much lower concentration factors (2-3 orders of magnitude for iodine, and < 1 order of magnitude for bromine) than e.g. in the kelp *Laminaria digitata*. Iodide competitively reduces the accumulation of bromide. Both iodide and bromide are accumulated in the cell wall (apoplast) of *Ectocarpus*, with minor amounts of bromine also detectable in the cytosol. *Ectocarpus* emits a range of volatile halogenated compounds, the most prominent of which by far is methyl iodide. Interestingly, biosynthesis of this compound cannot be accounted for by vanadium haloperoxidase since the latter have not been found to catalyze direct halogenation of an unactivated methyl group or hydrocarbon so a methyl halide transferase-type production mechanism is proposed.

Keywords Energy-dispersive X-ray analysis · Halocarbons · Methyl iodide · Phaeophyta · X-ray absorption spectroscopy

Abbreviations

CCAP	Culture Collection of Algae and Protozoa
DOM	Dissolved organic matter
EDX	Energy-dispersive X-ray analysis
EPR	Electron paramagnetic resonance
EXAFS	Extended X-ray absorption fine structure
GG	Oligo-guluronates
ICP-MS	Inductively coupled plasma-mass spectrometry
NIST	National Institute of Standards and Technology
PMA	Phorbol myristate acetate
vBPO	Vanadium bromoperoxidase
vIPO	Vanadium iodoperoxidase
VHPO	Vanadium haloperoxidase
XAS	X-ray absorption spectroscopy

This article is dedicated to the memory of Yasuyuki Muramatsu (January 9, 1950–July 2, 2016), a dear friend and outstanding scientist in the fields of Radioecology, Radiochemistry, Geochemistry and Analytical Chemistry in Japan, who untimely passed away during the writing of this article, and to Alison Butler (University of California, Santa Barbara) on the occasion of her Bader Award.

✉ Frithjof C. Küpper
fkuepper@abdn.ac.uk

[1] Oceanlab, University of Aberdeen, Main Street, Newburgh AB41 6AA, Scotland, UK

[2] Dunstaffnage Marine Laboratory, Scottish Association for Marine Science, Oban, Argyll PA37 1QA, Scotland, UK

[3] Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182-1030, USA

[4] Wolfson Atmospheric Chemistry Laboratories, Department of Chemistry, University of York, York YO10 5DD, UK

[5] Environment Department, University of York, York YO10 5NG, UK

[6] Department of Chemistry - Inorganic Chemistry, Faculty of Science, University of Paderborn, Warburger Strasse 100, 33098 Paderborn, Germany

[7] Geological Survey of Japan, The National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8567, Japan

[8] Department of Chemistry, Faculty of Science, Gakushuin University, Toshima-Ku, Tokyo 171-8588, Japan

[9] Department of Organic Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

Published online: 09 March 2018

Introduction

Two centuries ago, the elements bromine and iodine were discovered in sea water and seaweed (*Laminaria* and *Fucus*) ashes, respectively [1, 2]. Due to their unique evolutionary history and phylogenetic distance from other important eukaryotic lineages [3, 4], brown algae present some remarkable chemical and physiological adaptations which are also reflected at the genome level [5], making them fascinating experimental models, not only for phycologists, but for a community of interdisciplinary researchers. This includes the function of iodide as extracellular antioxidant protecting the surface of *Laminaria digitata* (Hudson) Lamouroux against oxidative stress [6]. Indeed, *L. digitata* is the strongest iodine accumulator currently known among living organisms [7]. In fact, this constituted the first documented case of an inorganic antioxidant in a living system—and the chemically simplest antioxidant known. Key to the antioxidant function is the mobilization of iodide in the apoplast and its efflux into the surrounding seawater upon oxidative stress [6, 8]. In this context, communities of the giant kelp *Macrocystis* have been found to impact iodine speciation in coastal sea water [9]. The role of bromine is less well investigated, but a recent study [10] highlights that *Laminaria* accumulates bromide, which complements iodide as an antioxidant especially for the detoxification of superoxide, but with an overall more diverse role.

In contrast to kelps such as *Laminaria* and *Macrocystis*, which have a large size (up to around 3 m for *Laminaria*, and 70 m for *Macrocystis*, respectively), very complex morphology and which can attain a high standing stock in rocky coastal seabed ecosystems, *Ectocarpus* is a genus of filamentous brown algae with a worldwide distribution along temperate coastlines, and is a nuisance as a "fouling" organism on many man-made surfaces in the sea. It has some significant advantages as an experimental model compared to *Laminaria* or *Macrocystis* and constitutes one of the best-studied seaweeds [11, 12]: due to its small size of only a few mm or cm and its fast growth it can easily be cultivated in small volumes of seawater media both axenically and with associated bacteria [13]; it belongs to a sister group of the ecologically and economically very important kelps [14]; its entire, well-known life cycle can be completed within a few months in culture, many molecular tools are available, including mutant collections, microarrays and proteomics. It has also become the first seaweed of which the entire genome has been sequenced and thus offers unprecedented opportunities for study [5]. Features of its inorganic biochemistry include a remarkable, non-ferritin mode of iron storage [15]. *Ectocarpus* is also well studied with regards to its pathologies which include viruses [16, 17], fungi [18], oomycetes [18] and plasmodiophoraleans [19].

While brown algae of the genus *Laminaria* are the strongest accumulators of iodine in life on Earth [20–22] and a major contributor to the biogeochemical flux of iodine [23] and, to a lesser extent, of brominated and iodinated halocarbons to the atmosphere [24], hardly anything is known about *Ectocarpus* in this respect. This is remarkable considering the major role of brown algae in global halogen cycling, but also how well other areas of physiology and biochemistry have been studied in *Ectocarpus*. However, a single gene of a vanadium haloperoxidase (VHPO) and a number of enzymes related to halogen metabolism have been identified in the *Ectocarpus* genome. This includes at least three different families (21 loci) of haloacid dehalogenase (HAD) and two haloalkane dehalogenase families [5, 25]. It is tempting to hypothesize that the dehalogenases protect *Ectocarpus* against halocarbons which act as alkylating, toxic agents and are being produced by kelps and other potential substrate algae on which *Ectocarpus* occurs. The presence of a single VHPO gene, a bromoperoxidase, in the *Ectocarpus* genome contrasts with the presence of multigenic families of VHPOs, of both bromo- and iodoperoxidases in the kelps *Laminaria* [26, 27] and *Macrocystis* [28].

The uptake of iodide from seawater in *Laminaria* involves VHPOs [21] and its strongest accumulation in this species seems to be linked to the presence of a particular VHPO subclass, the iodoperoxidases, specific for iodide oxidation [26, 27]. In *Laminaria*, most of the iodine is accumulated in the apoplast of the cortical cell layer [29].

The first line of defense against pathogens in *Laminaria* is an oxidative burst [30], which is considered a central element of eukaryotic defense in general [31] and which, in the case of kelp species, serves to control bacterial biofilms [32]. Among its triggers are oligoguluronates (GG; [30, 32]), bacterial lipopolysaccharides [33], prostaglandin A_2 [34], methyl jasmonate, and polyunsaturated free fatty acids [35]. In *L. digitata*, early transcriptional defense responses are similar to those in land plants but also involve tightly regulated halogen metabolism which might play roles in more sophisticated chemical defense reactions including distance signaling [36, 37]. In brown algae, bromide and VHPOs have also been shown to catalyze oxidative cross-linking between cell wall polymers, suggesting a role in spore and gamete adhesion and cell-wall strengthening (for reviews see [38, 39]). Much less is known about defense mechanisms in *Ectocarpus*. Recent studies observed strong differences in susceptibility to infection by *Eurychasma dicksonii* among different *Ectocarpus* strains [40], and *Eurychasma* infection [41] as well as Cu stress [42] were found to result in overexpression of the single VHPO encoded in the *Ectocarpus* genome.

In our previous studies [6, 10, 43], X-ray absorption spectroscopy (XAS) has proven to be a suitable, non-invasive tool to probe the chemical state and solution environment of accumulated iodine and bromine in kelps. Likewise, we have used energy-dispersive X-ray analysis (EDX) for localization studies of Fe in *Ectocarpus* [44].

Following our recent study which included *Ectocarpus* among a number of taxa from various algal lineages [43], this study explores the speciation and localization of bromine and iodine metabolism in *Ectocarpus*. First iodine and bromine levels in *Ectocarpus* are determined using ICP-MS. Then the localization of bromine and iodine is investigated using energy-dispersive analysis of X-rays. K-edge XAS is applied to probe the stored chemical state of these elements under different physiological conditions in vivo (both in healthy cultures and in the presence of the oomycete *Eurychasma dicksonii*). Finally, the production of volatile halocarbons is explored.

Materials and methods

Biological material

A well-characterized strain of *E. siliculosus* which had served for sequencing the *Ectocarpus* genome [5] was obtained from the Culture Collection of Algae and Protozoa (CCAP 1310/4) and was used for all experiments described here. *Ectocarpus* was grown in half-strength Provasoli-enriched sea water as described previously [13, 45, 46], i.e. at 18.

Eurychasma-infected, unialgal host cultures were grown as described previously [13, 46], i.e. at 15 °C, illumination by 20 to 30 µE m^{-2} s^{-1} from daylight type fluorescent lamps for 16 h day^{-1}. In a first experiment, about equal amounts (about 50 mg fresh weight each) of 6 *Ectocarpus* strains (Ec sil NZ Vic KU13/CCAP 1310/56, Ec fas Irl96 23-1n/CCAP 1310/342, Ec sil NZ4a3♀/CCAP 1310/47, Ec sil Vic88 12-1 lat. sp./CCAP 1310/185, Ec sil Vic Z2 lat. sp./CCAP 1310/191, Ec sil vic Z1 lat sp./CCAP 1310/192) infected by *Eurychasma* 96 [CCAP 4018/2, isolated from Shetland [18, 47] which had been cultured for about 6 months were pooled and used for XAS (below). In a second set of experiments, the following host–pathogen strain combinations were used: the host strains *E. siliculosus* (CCAP 1310/4, the genome model strain used by [5]), *E. siliculosus* (CCAP 1310/56, Ecsil NZ KU 1-3♂), *E. fasciculatus* (Ec fas Ros 007-04), *Pylaiella littoralis* (CCAP 1330/3, Pyl IR g), either without any *Eurychasma* infection, or infected with *E. dicksonii* Eury 96 (CCAP 4018/2) or *E. dicksonii* Eury 05 (CCAP 4018/1), respectively. Algal host strains (which included a close relative of *Ectocarpus*, *Pylaiella littoralis*) were initially cultured for 55 days on their own. Then, the above *Eurychasma* inocula were added and cultures were continued for another 30 days. Host–pathogen co-cultures were then harvested, placed in Kapton™ cells, and frozen in liquid nitrogen until use for XAS.

ICP-MS analysis of total iodine and bromine

Iodine and bromine contents in freeze-dried *Ectocarpus* filament samples were analyzed by ICP-MS after pyrohydrolysis separation as described previously [48, 49]. Concentrations obtained are given in ppm (i.e. µg halogen g^{-1} of freeze-dried material).

Energy-dispersive analysis of X-rays

Ectocarpus siliculosus was harvested from cultures prior to fixation, dehydration, and embedding. Filaments were fixed in a 0.1 M phosphate buffer solution containing 2% (w/v) paraformaldehyde, 1% (w/v) glutaraldehyde, and 1% (w/v) caffeine for 2 h. The fixed cells were then washed with 0.1 M phosphate buffer and dehydrated in successive ethanol baths of 30, 50, 75, 85, 95, and 100% (three times). The cells were then embedded in 1:1 (v/v) ethanol/LR White resin (LWR; EMS, Hatfield, PA, USA) for 3 h followed by 100% LWR overnight in gelatin capsules under vacuum. Sections of 3 µm were cut on a Leica EMUC6 microtome and deposited on glass slides. Slides were coated with platinum in a Quorum Technologies Q150T ES sputter coater. platinum-coated samples were analyzed under high vacuum in a Quanta 450 FEG environmental scanning electron microscope (ESEM) equipped with an Oxford Instruments INCA energy dispersive X-ray (EDX) microanalysis system.

X-ray fluorescence spectroscopy

Ectocarpus filaments were washed briefly with 100 µM ethylenediaminetetracetic acid (EDTA) followed by distilled water to mitigate calcium ion interference in the fluorescence spectrum. Washed filaments were placed on Ultralene™ film (Spex Sample Prep, USA), allowed to dry, and then placed in the GEOCARS X-ray beam of the Advanced Photon Source at Argonne National Laboratory (Lemont, IL).

The selected region of interest was rastered at a resolution of 2 µm with an X-ray beam energy of 5.0 keV, flux of 1.206×10^{12} photons s^{-1}, and dwell time of 20 ms $pixel^{-1}$. The resulting iodine $L_{\beta 1}$ X-ray fluorescence signal was mapped using the Larch Mapviewer™ software version 0.9.32 [50]. By virtue of the intensity and broadness of the Ca $K_{\beta 1}$ signal, calcium ion interference precluded mapping of both the iodine $L_{\alpha 1}$ and $L_{\beta 1}$ fluorescent signals from untreated *Ectocarpus* filaments. Only after EDTA washing was the I $L_{\beta 1}$ signal resolved from Ca $K_{\beta 1}$, while the I $L_{\alpha 1}$

remained confounded by calcium due to proximity of their respective fluorescent energies.

X-ray absorption spectroscopy

Bromine and iodine K-edge XAS measurements and extended X-ray absorption fine structure (EXAFS) data reduction were carried out at the EMBL Outstation Hamburg at DESY, Germany as described previously [51].

Halocarbons

Ectocarpus was sub-cultured into three 1 L flasks filled to 800 mL with sterilized coastal seawater enriched with Guillard's F/2. Three identical flasks of the media (excluding the *Ectocarpus* culture) were incubated as controls. Incubation conditions were 20 °C at 100 µmol m^{-2} s^{-1} and a 12:12 light/dark cycle. Flasks were stoppered with sterilized cotton in hessian plugs and covered with foil.

For *Ectocarpus* emission experiments, the solid contents of a culture flask was divided into two 200 mL flasks, filled completely to eliminate headspace with purged, filtered seawater obtained from the Atlantic Ocean at 3000 m depth and sealed with glass stoppers. A third flask was prepared without *Ectocarpus* addition as a control. The flasks were stored at 15 °C in the dark, sampled and immediately analyzed every 2 h over a 12 h period. 10 mL sample aliquots were taken each time using autoclaved perfluoroalkoxy (PFA) tubing into borosilicate gas syringes, filtered through a standard glass-fiber filter (GFF) and 10 mL of blank seawater added to compensate and eliminate the headspace. The blank seawater used was continuously purged throughout the course of the experiment to minimize VOC content. At the end of the 12 h period the flasks were filtered and the solid mass of *Ectocarpus* recorded. This was then allowed to air dry and the dry weight was recorded. For investigating the effects of oxidative stress, the experiment was performed as described above except for the addition of 2 mM H_2O_2 to simulate oxidative stress.

10 mL sample aliquots were analysed using purge and trap coupled to thermal desorption–gas chromatography—time of flight mass spectrometry (P&T-TD-GC-TOFMS, Markes Unity2 CIA8 TDU, Agilent 6890, Markes Bench-Tof MS) with a custom built purge and trap unit [52]. The TOF–MS allowed high sensitivity monitoring of all ions from 35 to 500 amu simultaneously. Halocarbons were calibrated using NOAA standard SX-3570, accounting for the purge and trap efficiencies of each gas [52]. The control flask was analysed first at each time point to check for carry-over in the analysis and/or production in the blank seawater. No significant change in the analysed VOC content of the blank seawater was observed throughout the emission experiments.

Results

Total halogen levels (ICP-MS)

Initially, halogen levels in cultured *Ectocarpus* were determined in order to establish whether there is any significant accumulation of iodine and bromine in this organism. Freeze-dried thalli of the brown alga *Ectocarpus siliculosus* cultivated in normal PES medium contained mean concentrations of 196 ± 11 ppm iodine and 738 ± 3 ppm bromine. In contrast, freeze-dried *Ectocarpus siliculosus* thalli cultivated in PES medium supplemented with 50 µM KI contained 804 ± 3 ppm iodine, but only 186 ± 3 ppm bromine.

Localization of halogens (EDX and XFS)

Next, the localization of halogens was explored in order to get first insight into potential function. Based on XFS and EDX, most iodine was concentrated in physode-like vesicles (Fig. 1), even though some was also detectable in the cytosol and/or apoplast (Fig. 2). The EDX signal from bromine (Fig. 2) was considerably weaker than that of iodine, showing some localization to the apoplast, cytosol, and possibly intracellular structures (Fig. 2).

XAS

XAS is the method of choice for determining the chemical environment and redox state of halogens in seaweeds [51]. The X-ray Absorption Near-Edge spectra (XANES) at the Br K-edge in Fig. 3 show that non-infected *Ectocarpus* (trace C) is quite similar to an aqueous solution of NaBr (trace D); it appears that Br is present in *Ectocarpus* as hydrated bromide [43]. The spectrum of *Ectocarpus siliculosus* infected with *Eurychasma dicksonii* (trace B) is subtly different and resembles more that of Br incorporated in an aromatic system, such as in the model compound 4-bromophenylalanine (trace A). The spectra show that in uninfected algae, bromine is present in its reduced anionic form, bromide, whereas upon infection prevailing over several months, incorporation of a major fraction into organic compounds occurs, concomitant with general senescence of the cultures. However, this was not detectable in experiments with a shorter time span of infective pressure.

It was shown earlier [43, 51] that the Extended X-ray Absorption Fine Structure (EXAFS) of hydrated bromide could be simulated with a shell of oxygen atoms at approx. 3.3 Å; although the hydrogen atoms cannot be observed in EXAFS, it can be safely assumed that these oxygens are non-covalently bound to bromide by hydrogen bonds. For non-infected *Ectocarpus* this is the only contribution (Fig. 4a, b, bottom trace in both panels, attempts to simulate the weaker

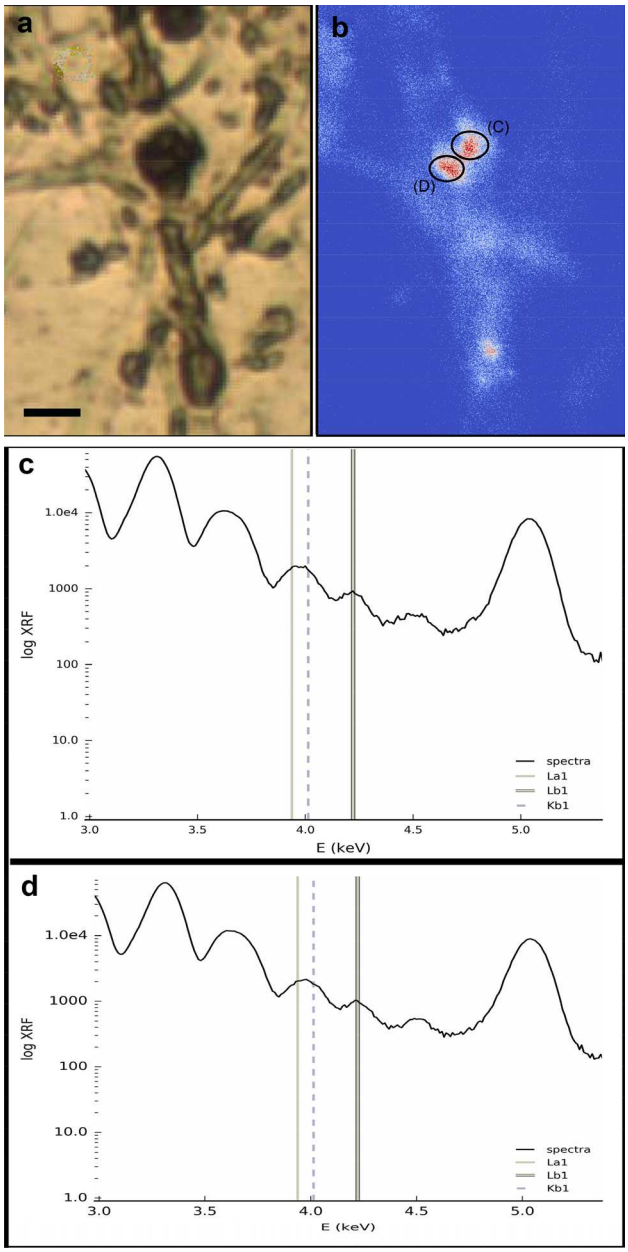

Fig. 1 **a** Optical image of *Ectocarpus* filaments; **b** heat map of iodine $L_{\beta 1}$ fluorescence intensity with two regions of interest showing high iodine concentrations; **c**, **d** fluorescence spectra for regions of interest from **b**. Light and dashed vertical lines denote I $L_{\alpha 1}$ and Ca $K_{\beta 1}$ signals, respectively. Dark vertical lines represent the I $L_{\beta 1}$ signal. These are whole, intact filaments (not a section). The two circled regions of interest are probably physodes at the junction of the main filament and a branched filament. Scale bar = 10 µm

peaks in the Fourier transform did not lead to significant improvements). For infected *Ectocarpus* (Fig. 4a, b, top traces in both panels) the carbon peak in the Fourier transform is the strongest, and the pattern of peaks is reminiscent of that of aromatic amino acids such as 4-bromophenylalanine and 3,5-dibromotyrosine. The Br–C distance found in the simulation with both a phenyl group and a shell of H-bonded heteroatoms is 1.90 Å, which is in between the ranges considered typical for bonds with sp^2 (1.87–1.88 Å) and sp^3 (1.92–1.96 Å) C; the good agreement between experiment and simulation for the other atoms of the phenyl ring strongly indicates sp^2 C in this case (Table 1). For the incorporation of Br in organic compounds to be observed by XAS, it is necessary that the infection lasts for a prolonged duration of time (> 1 month in a batch culture); experiments in which the infection was left for only 3 weeks did not result in EXAFS other than that of hydrated bromide.

The iodine K-edge spectra (Fig. 5) showed hardly any characteristic features. The EXAFS was so weak that it could not be reliably extracted. The XANES of non-infected *Ectocarpus* (Fig. 5, trace C) remotely resembles that of an aqueous solution of NaI (trace E) and probably represents iodide in the form of I^-. In its lack of features it resembles the XANES of lyophilized *Laminaria* [6] which contains iodide surrounded by a rather disordered shell of hydrogen-bonded heteroatoms, probably from biomolecules rather than water. Addition of 50 µM iodate (trace B) resulted in an increase of the edge step by a factor of approx. 2 prior to normalization (not shown). This addition did however not result in a significant change in the appearance of the edge, as might have been expected on the basis of the spectrum of pure iodate (trace A), suggesting that the added iodate is reduced to iodide upon contact with *Ectocarpus*. Addition of 50 µM iodide (trace D) resulted in a much larger edge step prior to normalization (not shown), viz. by a factor of 5. The resulting spectrum is featureless like that of the control, except for the more pronounced shoulder at 33,230 eV. In spite of the addition of a considerable amount of hydrated iodide, the features are not as sharp as that of the NaI solution (trace E). This implies that the iodide was taken up by *Ectocarpus* and that a relatively ordered hydration shell was replaced by biomolecules.

Emission of volatile compounds

In virtually all other seaweed species studied, halogen metabolism comes with the emission of volatile halogenated compounds [7, 24]. In the present study of *Ectocarpus*, the most predominant emission from the list of calibrated species (CH_3I, CH_2BrCl, $CHCl_3$, CCl_4, CH_2Br_2, $CHBrCl_2$, CH_2ICl, $CHBr_2Cl$, CH_2IBr, $CHBr_3$) was methyl iodide (CH_3I), which was markedly enhanced under oxidative stress conditions with a production rate of 1.16×10^{-5} pmol (g FW × min)$^{-1}$ when exposed to 2 mM H_2O_2 compared to 5.98×10^{-6} pmol (g FW × min)$^{-1}$ under control conditions during the first 2 h of the experiment (after which production leveled off, cf. Fig. 6). The % relative

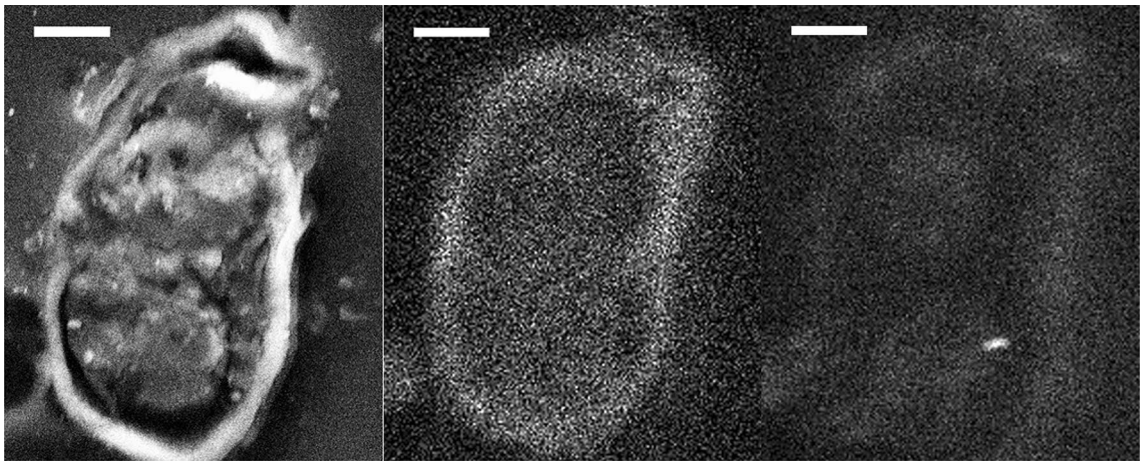

Fig. 2 SEM image of an *E. siliculosus* section (left), EDX map of iodine (middle), and EDX map of bromine (right). Scale bar = 5 µm. This is a cross-section of a filament that had been fixed, dehydrated, embedded in acrylic, sectioned with a microtome, and coated with platinum before performing EDX

standard deviation for CH_3I for this system is 9.4% [52]. No appreciable production of bromocarbons was observed. Many other non-halogenated species, mostly oxygenated, were detected (but calibrations were not available) based on similarities with NIST (National Institute of Standards and Technology) Mass Spectral Library spectra including acetone, isopropyl alcohol, 2-methyl propanol, butanal, 2-butanone, hexane, 3-methyl 2-butanone, 2-pentanone, pentanal, 3-methyl 2-pentanone, 3-methyl pentanal, 2-hexanone, hexanal, 4-methyl 2-hexanone, 3-heptanone, heptanal, 2-methyl 4-heptanone, 3-methyl 4-heptanone, 4-methyl 2-heptanone, 5-methyl 3-heptanone, 6-methyl 2-heptanone, 5-methyl 2-heptanone and 2-octanone.

Discussion

Overall, the results of this study show that *Ectocarpus* has an active bromine and iodine metabolism. Both elements are actively accumulated from surrounding seawater, albeit (especially in the case of iodine) at much lower concentration factors than *Laminaria* [20, 21]: This study observed concentration factors of 714 for iodine and 2.6 for bromine (i.e. 2–3 and < 1 order of magnitude, respectively, based on fresh weight) compared to normal seawater. This compares to up to 5 orders of magnitude for iodine [21] and around 1 order of magnitude for bromine in *Laminaria* [10], and 3–4 orders of magnitude for iodine in *Macrocystis* [28]. Interestingly, artificial iodine supplementation (50 µM KI) in the culture medium resulted in a much lower bromine tissue concentration (186 ± 3 ppm) than in *Ectocarpus* cultures grown in medium without iodide supplementation (738 ± 3 ppm). This suggests that iodide and bromide compete for the same uptake and storage system, in which the sole V-bromoperoxidase detected in *Ectocarpus* [5, 25] is likely the key driver. Iodide is well established as being the preferred substrate of V haloperoxidases [53], viz. of both bromo- and iodoperoxidases [27]. Our recent work had already shown that *Ectocarpus* accumulates bromide [43], similar to morphologically more complex brown algae (Laminariales and Fucales). Here, XAS has shown that, like *Laminaria* [6], *Ectocarpus* also accumulates iodine in its reduced form, iodide, bound to H-bonding heteroatoms (O–H, N–H) in biomolecules, with the hydration shell removed, as in lyophilized *Laminaria*. Again, similar to *Laminaria* [29], the present study also shows that *Ectocarpus* accumulates bromide and iodide in the apoplast (iodine storage is exclusively apoplastic, while smaller amounts of bromine can also be detected in the cytosol—Fig. 1). Our observation that the addition of iodate to the *Ectocarpus* growth medium results in an increased (estimated from X-ray absorption intensity) uptake of the element as iodide implies a reduction, which is in line with recent observations concerning the iodine uptake by marine macroalgae [9]. Interestingly, the reductant could be iodide, to form elemental iodine, which may be considered besides HOI to be the neutral species that is formed as an intermediate in VHPO-mediated iodine uptake. According to Küpper & Kroneck [54] the equilibrium is towards disproportionation at high pH and temperature, so that one might argue that starting at lower T and pH with only IO_3^- and I^-, some I_2 might be formed by comproportionation, and removed from the equilibrium by uptake in *Ectocarpus* and subsequent reduction, potentially explaining the enhanced iodine uptake upon addition of iodate.

The strong production of methyl iodide (CH_3I), which was slightly increased under oxidative stress conditions,

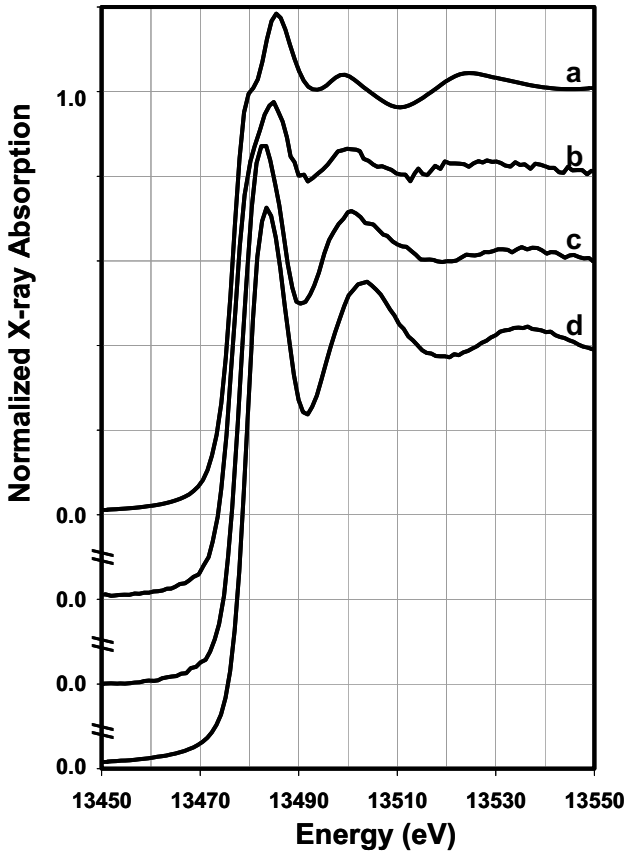

Fig. 3 Br K-edge XANES. Bromine K-edge X-ray absorption edge spectra of *Ectocarpus* and relevant model compounds: **a** 4-bromophenylalanine (aq., from [51]); **b** *Ectocarpus siliculosus* infected with *Eurychasma dicksonii*; **c** non-infected *Ectocarpus siliculosus*, and **d** NaBr (10 mM, aq., from [51])

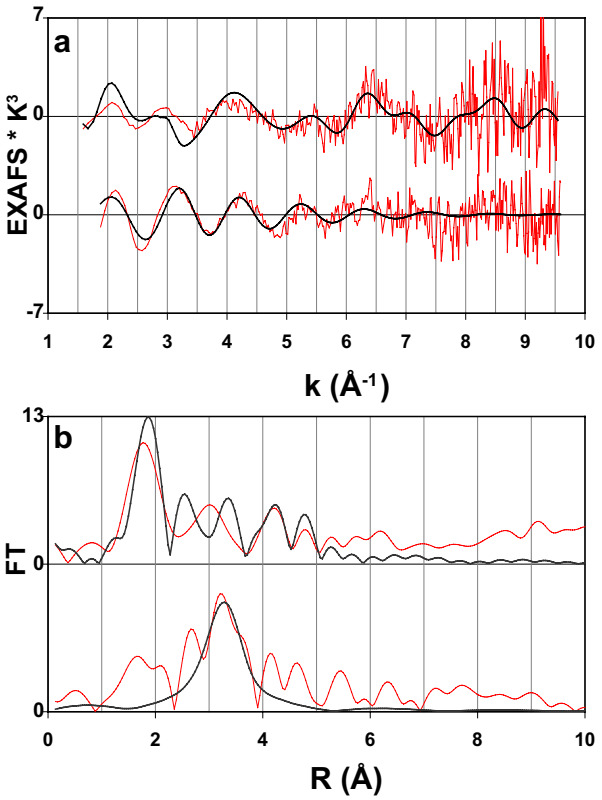

Fig. 4 Experimental (red) and simulated (black) k^3-weighted Br K-edge EXAFS (**a**) and its Fourier transform (**b**) of (top traces) *Ectocarpus siliculosus* infected with *Eurychasma dicksonii*, and (bottom traces) non-infected *Ectocarpus siliculosus*. Simulation parameters are given in Table 1

is remarkable and stands out among macroalgae. Production of this compound cannot be accounted for by the V bromoperoxidase present in *Ectocarpus* (which can, at least in theory, account for the production of CH_2BrCl, $CHCl_3$, CCl_4, CH_2Br_2, $CHBrCl_2$, CH_2ICl, $CHBr_2Cl$, CH_2IBr, $CHBr_3$), catalysis of which results in compounds with multiple halogenations on the same carbon atom. It is worth noting that some of the methyl ketones observed here (2-butanone, 3-methyl 2-butanone, 2-pentanone, 3-methyl 2-pentanone, 2-hexanone, 4-methyl 2-hexanone, 4-methyl 2-heptanone, 6-methyl 2-heptanone, 5-methyl 2-heptanone and 2-octanone) would be good starting materials for the haloform reaction [55, 56] that we proposed recently [43], which could account for the trihalomethanes listed above.

Instead, it is tempting to hypothesize that an *S*-adenosylmethionine-dependent methyl transferase-type mechanism is operative here, similar to that described for methyl halide transferase, a class of enzymes which is responsible for chloromethane production in the higher plant *Mesembryanthemum crystallinum* [57] and other salt-tolerant higher plants [58]. Considering that halocarbon production in seaweeds has usually been associated with oxidative stress and, in

Table 1 EXCURVE results for bromine K-edge EXAFS (Fig. 4, Debye–Waller factors as $2\sigma^2$ (Å^2) in parentheses)

Ectocarpus siliculosus infected with *Eurychasma dicksonii*	Fit index 0.3985E−02 1.00 Ph (1 C at 1.89940 Å (0.00916); 2 C at 2.74504 Å (0.01912); 2 C at 4.28701 Å (0.02146); 1 C at 4.71337 Å (0.00297) + 1.43 O at 3.38615 Å (0.00302) EF − 11.0613 eV, range 4.3623–350 eV	Br^- + sp^2 C
Ectocarpus siliculosus	Fit index 0.3011E−02 7.53 O at 3.36691 Å (0.05396) EF − 8.4995 eV, range 4.5913–350 eV	Br^-

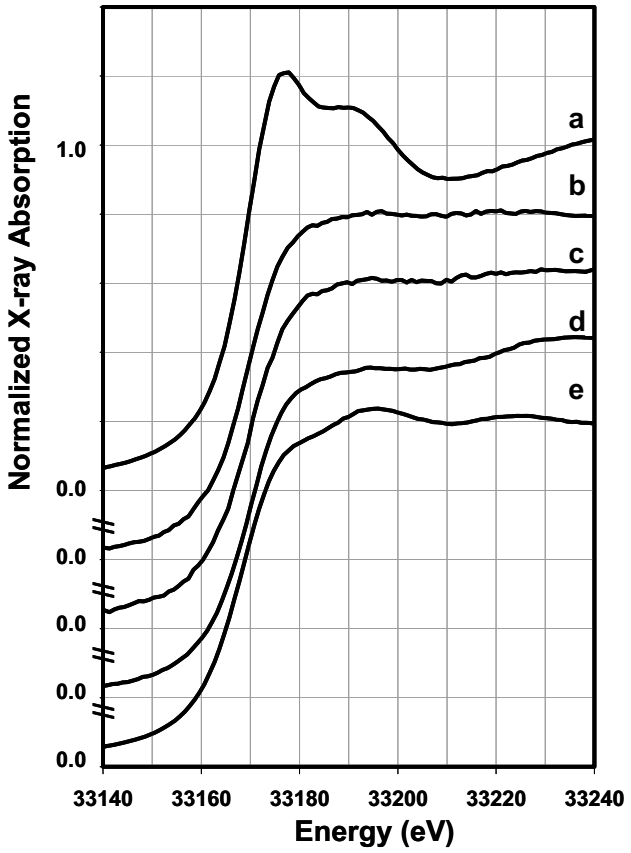

Fig. 5 I K-edge XANES. Iodine K-edge X-ray absorption edge spectra of *Ectocarpus* and relevant model compounds: **a** $NaIO_3$ (aq., from [51]); **b** *Ectocarpus siliculosus* with 50 µM $NaIO_3$ added; **b**, **c** *Ectocarpus siliculosus* control; **d** *Ectocarpus siliculosus* with 50 µM NaI added; and **e** NaI (20 mM, aq., from [51])

particular, with hydrogen peroxide, it has to be highlighted that this reaction is independent of any reactive oxygen species:

S-adenosyl-L-methionine + halide →

S-adenosyl-L-homocysteine + methyl halide.

It should be noted that (like for *V* haloperoxidases), iodide is the preferred substrate for methyl halide transferases, with usually much lower K_M and higher V_{max} values reported for iodide over bromide and chloride [58]. While a very moderate increase of methyl iodide production was observed in this study under oxidative stress conditions, this may arguably not be due to the direct participation of H_2O_2 in the biosynthetic reaction(s), but rather due to the mobilization of iodide from its apoplastic store due to H_2O_2, analogous to the situation in *Laminaria* [6], thus merely increasing substrate availability for a hypothetical methyl transferase-type enzyme. It should be noted that *Ectocarpus* genome annotation has at present not uncovered any *S*-adenosyl-methionine-dependent methyl transferase—but also that, at present, many open reading frames in the *Ectocarpus* genome

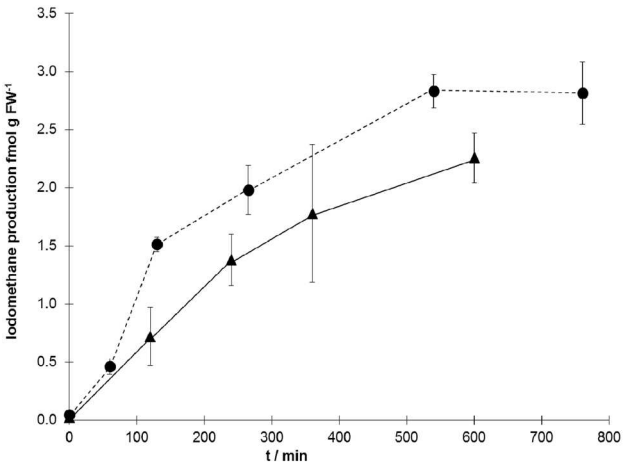

Fig. 6 Iodomethane production by *E. siliculosus* without (triangles) and with (circles) 2 mM H_2O_2 treatment

encode for genes of still unknown function [25]. A BLAST search of the complete cDNA sequence of *Beta maritima* [AF084829; 58] against the *Ectocarpus* genome (11×) and latest (as of November 2017) cDNA database did not yield any significant matches (not shown). Other halogen-related enzymes that have been identified in the *Ectocarpus* genome include at least three different families (21 loci) of haloacid dehalogenase (HAD) and two haloalkane dehalogenases. The HADs belong to a large superfamily of hydrolases with diverse substrate specificity, including phosphatases and ATPases. The dehalogenase enzymes may contribute to protect *Ectocarpus* against halogenated compounds produced as defence metabolites by kelps [6] allowing it to successfully grow as an epiphyte or endophyte on kelp thalli [59, 60].

Acknowledgements Funding from the UK Natural Environment Research Council (NERC) through grants NE/D521522/1 (FCK), NE/F012705/1 (FCK), NE/K000454/1 (CH), and the Oceans 2025 (WP4.5) program FCK; the National Science Foundation (CHE-1664657) to CJC; and the MASTS pooling initiative (Marine Alliance for Science and Technology for Scotland, funded by the Scottish Funding Council and contributing institutions; grant reference HR09011) is gratefully acknowledged. We are also grateful to Claire M.M. Gachon (Scottish Association for Marine Science) for her assistance with the *Ectocarpus*/*Eurychasma* cultures. EPM and CJC are grateful to the Advanced Photon Source at Argonne National Laboratory for facilitating the GSECARS X-ray experiments and Tony Lanzirotti for his assistance on the GSECARS beamline and with subsequent data analysis. Furthermore, the authors are grateful for support from the European Community in the framework of the Access to Research Infrastructure Action of the Improving Human Potential Program to the EMBL Hamburg Outstation. Finally, we would like to thank Yuka Uchida (Department of Chemistry, Gakushuin University, Tokyo) for her help with the ICP-MS analyses mentioned here.

References

1. Balard AJ (1826) Ann Chim Phys (Paris) 32:337–384
2. Gay-Lussac L-J (1813) Ann Chim (Paris) 88:311–318
3. Baldauf SL (2003) Science 300:1703–1706
4. Bhattacharya D, Stickel SK, Sogin ML (1991) J Mol Evol 33:525–536
5. Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury J-M, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JHF, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collén J, Corre E, Da Silva C, Delage L, Delaroque N, Dittami SM, Doulbeau S, Elias M, Farnham G, Gachon CMM, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Küpper FC, Lang D, Le Bail A, Leblanc C, Lerouge P, Lohr M, Lopez PJ, Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli CA, Nelson DR, Nyvall-Collén P, Peters AF, Pommier C, Potin P, Poulain P, Quesneville H, Read B, Rensing SA, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder DC, Ségurens B, Strittmatter M, Tonon T, Tregear J, Valentin K, von Dassow P, Yamagishi T, Van de Peer Y, Wincker P (2010) Nature 465:617–621
6. Küpper FC, Carpenter LJ, McFiggans GB, Palmer CJ, Waite TJ, Boneberg EM, Woitsch S, Weiller M, Abela R, Grolimund D, Potin P, Butler A, Luther GW III, Kroneck PMH, Meyer-Klaucke W, Feiters MC (2008) Proc Natl Acad Sci USA 105:6954–6958
7. Küpper FC, Feiters MC, Olofsson B, Kaiho T, Yanagida S, Zimmermann MB, Carpenter LJ, Luther GW III, Lu Z, Jonsson M, Kloo L (2011) Angew Chem Int Ed 50:11598–11620
8. Chance R, Baker AR, Küpper FC, Hughes C, Kloareg B, Malin G (2009) Estuar Coast Shelf Sci 82:406–414. https://doi.org/10.1016/j.ecss.2009.02.004
9. Gonzales J, Tymon T, Küpper FC, Edwards MS, Carrano CJ (2017) PLoS ONE 12:e0180755. https://doi.org/10.1371/journal.pone.0180755
10. Küpper FC, Carpenter LJ, Leblanc C, Toyama C, Uchida Y, Verhaeghe E, Maskrey B, Robinson J, Boneberg E-M, Malin G, Luther GW III, Kroneck PMH, Kloareg B, Meyer-Klaucke W, Muramatsu Y, Potin P, Megson IL, Feiters MC (2013) J Exp Bot 64:2653–2664
11. Peters AF, Marie D, Scornet D, Kloareg B, Cock JM (2004) J Phycol 40:1079–1088
12. Charrier B, Coelho SM, Le Bail A, Tonon T, Michel G, Potin P, Kloareg B, Boyen C, Peters AF, Cock JM (2008) New Phytol 177:319–332
13. Müller DG, Gachon CMM, Küpper FC (2008) Cah Biol Mar 49:59–65
14. Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, de Reviers B, Rousseau F (2010) Mol Phylogenet Evol 56:659–674. https://doi.org/10.1016/j.ympev.2010.04.020
15. Böttger LH, Miller EP, Andresen C, Matzanke BF, Küpper FC, Carrano CJ (2012) J Exp Bot 63:5763–5772
16. Müller DG, Kapp M, Knippers R (1998) Adv Virus Res 50:49–67
17. Müller DG, Kawai H, Stache B, Lanka S (1990) Bot Acta 103:72–82
18. Müller DG, Küpper FC, Küpper H (1999) Phycol Res 47:217–223
19. Maier I, Parodi E, Westermeier R, Müller DG (2000) Protist 151:225–238
20. Ar Gall E, Küpper FC, Kloareg B (2004) Bot Mar 47:30–37
21. Küpper FC, Schweigert N, Ar Gall E, Legendre JM, Vilter H, Kloareg B (1998) Planta 207:163–171
22. Saenko GN, Kravtsova YY, Ivanenko VV, Sheludko SI (1978) Mar Biol 47:243–250
23. McFiggans GB, Coe H, Burgess R, Allan J, Cubison M, Alfarra MR, Saunders R, Saiz-Lopez A, Plane JMC, Wevill DJ, Carpenter LJ, Rickard AR, Monks PS (2004) Atmos Chem Phys 4:701–713
24. Carpenter LJ, Malin G, Küpper FC, Liss PS (2000) Glob Biogeochem Cycles 14:1191–1204
25. Cock JM, Sterck L, Ahmed S, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Arun A, Aury JM, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JH, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collen J, Le Corguille G, Corre E, Dartevelle L, Da Silva C, Delage L, Delaroque N, Dittami SM, Doulbeau S, Elias M, Farnham G, Gachon CMM, Godfroy O, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Kuepper FC, Lang D, Le Bail A, Luthringer R, Leblanc C, Lerouge P, Lohr M, Lopez PJ, Macaisne N, Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli CA, Nelson DR, Nyvall-Collen P, Peters AF, Pommier C, Potin P, Poulain J, Quesneville H, Read B, Rensing SA, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder DC, Scornet D, Segurens B, Strittmatter M, Tonon T, Tregear JW, Valentin K, Von Dassow P, Yamagishi T, Rouze P, Van de Peer Y, Wincker P, Ectocarpus Genome C (2012) In: Piganeau G (ed) Genomic insights into the biology of algae. Academic Press Ltd-Elsevier Science Ltd, London, pp 141–184
26. Colin C, Leblanc C, Michel G, Wagner E, Leize-Wagner E, van Dorsselaer A, Potin P (2005) J Biol Inorg Chem 10:156–166
27. Colin C, Leblanc C, Wagner E, Delage L, Leize-Wagner E, Van Dorsselaer A, Kloareg B, Potin P (2003) J Biol Chem 278:23545–23552
28. Tymon TM, Miller EP, Gonzales JL, Raab A, Küpper FC, Carrano CJ (2017) J Inorg Biochem 177:82–88. https://doi.org/10.1016/j.jinorgbio.2017.09.003
29. Verhaeghe EF, Fraysse A, Guerquin-Kern J-L, Wu T-D, Devès G, Mioskowski C, Leblanc C, Ortega R, Ambroise Y, Potin P (2008) J Biol Inorg Chem 13:257–269
30. Küpper FC, Kloareg B, Guern J, Potin P (2001) Plant Physiol 125:278–291
31. Wojtaszek P (1997) Biochem J 322:681–692
32. Küpper FC, Müller DG, Peters AF, Kloareg B, Potin P (2002) J Chem Ecol 28:2057–2081
33. Küpper FC, Gaquerel E, Boneberg E-M, Morath S, Salaün J-P, Potin P (2006) J Exp Bot 57:1991–1999
34. Zambounis A, Gaquerel E, Strittmatter M, Potin P, Küpper FC (2012) Algae 27:1–12
35. Küpper FC, Gaquerel E, Cosse A, Adas F, Peters AF, Müller DG, Kloareg B, Salaün JP, Potin P (2009) Plant Cell Physiol 50:789–800. https://doi.org/10.1093/pcp/pcp023
36. Cosse A, Potin P, Leblanc C (2009) New Phytol 182:239–250. https://doi.org/10.1111/j.1469-8137.2008.02745.x
37. Thomas F, Cosse A, Goulitquer S, Raimund S, Morin P, Valero M, Leblanc C, Potin P (2011) PLoS One 6(6):e21475. https://doi.org/10.1371/journal.pone.0021475
38. La Barre S, Potin P, Leblanc C, Delage L (2010) Mar Drugs 8:988–1010. https://doi.org/10.3390/md8040988
39. Potin P, Leblanc C (2006) In: Smith AM, Callow JA (eds) Biological Adhesives. Springer Verlag, pp. 105-124
40. Gachon CMM, Strittmatter M, Müller DG, Kleinteich J, Küpper FC (2009) Appl Environ Microbiol 75:322–328
41. Strittmatter M, Grenville-Briggs LJ, Breithut L, van West P, Gachon CMM, Kupper FC (2016) Plant Cell Environ 39:259–271. https://doi.org/10.1111/pce.12533

42. Ritter A, Umbertini M, Romac S, Gaillard F, Delage L, Mann A, Cock JM, Tonon T, Correa JA, Potin P (2010) Proteomics 10:2074–2088
43. Küpper FC, Leblanc C, Meyer-Klaucke W, Potin P, Feiters MC (2014) J Phycol 50:652–664
44. Miller EP, Böttger LH, Weerasinghe AJ, Crumbliss AL, Matzanke BF, Meyer-Klaucke W, Küpper FC, Carrano CJ (2014) J Exp Bot 65:585–594. https://doi.org/10.1093/jxb/ert406
45. Starr RC, Zeikus JA (1987) J Phycol 23((Suppl.)):1–47
46. Strittmatter M, Grenville-Briggs LJ, Breithut L, Van West P, Gachon CMM, Küpper FC (2016) Plant. Cell Environ 39:259–271. https://doi.org/10.1111/pce.12533
47. Küpper FC, Müller DG (1999) Nova Hedwig 69:381–389
48. Chai JY, Muramatsu Y (2007) Geostand Geoanal Res 31:143–150. https://doi.org/10.1111/j.1751-908X.2007.00856.x
49. Schnetger B, Muramatsu Y (1996) Analyst 121:1627–1631. https://doi.org/10.1039/an9962101627
50. Newville M (2013) J Phys Conf Ser 430:012007. https://doi.org/10.1088/1742-6596/430/1/012007
51. Feiters MC, Küpper FC, Meyer-Klaucke W (2005) J Synchrotron Rad 12:85–93
52. Andrews SJ, Hackenberg SC, Carpenter LJ (2015) Ocean Sci 11:313–321. https://doi.org/10.5194/os-11-313-2015
53. Vilter H (1995) In: Sigel A, Sigel H (eds) Vanadium and its role in life. Marcel Dekker, New York, pp 325–362
54. Küpper FC, Kroneck PMH (2015) In: Kaiho T (ed) Iodine chemistry and applications. Wiley, Hoboken, pp 557–589
55. Theiler R, Cook JC, Hager LP, Studa JF (1978) Science 202:1094–1096
56. Beissner RS, Guilford WJ, Coates RM, Hager LP (1981) Biochemistry 20:3724–3731. https://doi.org/10.1021/bi00516a009
57. Wuosmaa AM, Hager LP (1990) Science 249:160–162. https://doi.org/10.1126/science.2371563
58. Ni X, Hager LP (1999) Proc Natl Acad Sci 96:3611–3615. https://doi.org/10.1073/pnas.96.7.3611
59. Russell G (1983) Mar Ecol Prog Ser 13:303–304. https://doi.org/10.3354/meps013303
60. Russell G (1983) Mar Ecol Prog Ser 11:181–187. https://doi.org/10.3354/meps011181