Journal of Experimental Botany, Vol. 64, No. 10, pp. 2653–2664, 2013
doi:10.1093/jxb/ert110 Advance Access publication 18 April, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

In vivo speciation studies and antioxidant properties of bromine in *Laminaria digitata* reinforce the significance of iodine accumulation for kelps

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Received 16 January 2013; Revised 7 March 2013; Accepted 19 March 2013

Abstract

The metabolism of bromine in marine brown algae remains poorly understood. This contrasts with the recent finding that the accumulation of iodide in the brown alga *Laminaria* serves the provision of an inorganic antioxidant – the first case documented from a living system. The aim of this study was to use an interdisciplinary array of techniques to study the chemical speciation, transformation, and function of bromine in *Laminaria* and to investigate the link between bromine and iodine metabolism, in particular in the antioxidant context. First, bromine and iodine levels in different *Laminaria* tissues were compared by inductively coupled plasma MS. Using *in vivo* X-ray absorption spectroscopy, it was found that, similarly to iodine, bromine is predominantly present in this alga in the form of bromide, albeit at lower concentrations, and that it shows similar behaviour upon oxidative stress. However, from a thermodynamic and kinetic standpoint, supported by *in vitro* and reconstituted *in vivo* assays, bromide is less suitable than iodide as an antioxidant against most reactive oxygen species except superoxide, possibly explaining why kelps prefer to accumulate iodide. This constitutes the first-ever study exploring the potential antioxidant function of bromide in a living system and other potential physiological roles. Given the tissue-specific differences observed in the content and speciation of bromine, it is concluded that the bromide uptake mechanism is different from the vanadium

iodoperoxidase-mediated uptake of iodide in *L. digitata* and that its function is likely to be complementary to the iodide antioxidant system for detoxifying superoxide.

Key words: Antioxidant, brown algae, electron paramagnetic resonance, halocarbons, reactive oxygen species, X-ray absorption spectroscopy.

Introduction

Two centuries ago, the elements bromine and iodine were discovered in seawater and seaweed ashes, respectively (Balard, 1826; Gay-Lussac, 1813). Due to their unique evolutionary history and phylogenetic distance from other important eukaryotic lineages (Bhattacharya *et al.*, 1991; Baldauf, 2003), brown algae present some remarkable chemical and physiological adaptations which are also reflected at the genome level (Cock *et al.*, 2010), making them fascinating experimental models, not only for phycologists, but for a community of interdisciplinary researchers. This includes the recently described function of iodide as an extracellular antioxidant protecting the surface of *Laminaria digitata* (Hudson) Lamouroux against oxidative stress (Küpper *et al.*, 2008). In fact, this constituted the first documented case of an inorganic antioxidant in a living system – and the chemically simplest antioxidant known.

Indeed, brown algae of the genus *Laminaria* are the strongest accumulators of iodine in life on Earth (Saenko *et al.*, 1978; Küpper *et al.*, 1998; Ar Gall *et al.*, 2004), and they are a major contributor to the biogeochemical flux of iodine (McFiggans *et al.*, 2004) and, to a lesser extent, brominated and iodinated halocarbons to the atmosphere (Carpenter *et al.*, 2000). However, while the uptake, metabolism, and biogeochemical cycling of iodine by *Laminaria* are well studied (Küpper *et al.*, 2011), hardly anything is known about bromine in this context. The uptake of iodide from seawater in *Laminaria* involves vanadium haloperoxidases (VHPOs) (Küpper *et al.*, 1998) 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 (Colin *et al.*, 2003, 2005). Most of the iodine is accumulated in the apoplast of the cortical cell layer (Verhaeghe *et al.*, 2008b). Following a hypothesis from the biomedical field about a potential ancestral role of iodide as an antioxidant in the evolution of the thyroid in vertebrates (Venturi and Venturi, 1999), it was shown that iodide is the accumulated form of iodine in *Laminaria*, and that it indeed serves as a simple, inorganic antioxidant, protecting the apoplast and thallus surface against both aqueous and gaseous oxidants (Küpper *et al.*, 2008). This study also revealed that the iodide in the liquid microlayer on the emerged *Laminaria* thallus surface can efficiently scavenge ozone from the gas phase, resulting in atmospheric particle formation, which can act as cloud condensation nuclei (McFiggans *et al.*, 2004; Palmer *et al.*, 2005). In this study, 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. However, this technique is equally suitable to investigate the speciation of bromine in kelps (Feiters *et al.*, 2005a,b; Strange and Feiters, 2008).

The first line of defence against pathogens in *Laminaria* is an oxidative burst (Küpper *et al.*, 2001), which is considered a central element of eukaryotic defence in general (Wojtaszek, 1997) and which, in the case of kelp species, serves to control bacterial biofilms (Küpper *et al.*, 2002). Among its triggers are oligoguluronates (GG) (Küpper *et al.*, 2001, 2002), bacterial lipopolysaccharides (Küpper *et al.*, 2006), prostaglandin A_2 (Zambounis *et al.*, 2012), methyl jasmonate, and polyunsaturated free fatty acids (Küpper *et al.*, 2009). In *L. digitata*, early transcriptional defence responses are similar to those in land plants but also involve tightly regulated halogen metabolism which might play roles in more sophisticated chemical defence reactions including distance signalling (Cosse *et al.*, 2009; Thomas *et al.*, 2011). In brown algae, bromide and VHPOs have also been shown to catalyse oxidative cross-linking between cell-wall polymers, suggesting a role in spore and gamete adhesion and cell-wall strengthening (for reviews, see Potin and Leblanc, 2006; La Barre *et al.*, 2010).

This study addresses the biological significance of bromine versus iodine metabolism in *Laminaria*. First, iodine and bromine levels in different types of tissues of *Laminaria* were investigated using inductively coupled plasma (ICP) MS. Then K-edge XAS was applied to probe the stored chemical state of these elements under different physiological conditions *in vivo* (both steady-state unstressed and oxidatively stressed) in the different tissue types of *Laminaria* and to draw comparisons and potential functional links between bromine and iodine metabolism. Finally, the potential antioxidant properties of bromide and iodide were compared in whole-blood oxidative burst assays and *in vitro* electron paramagnetic resonance (EPR) assays.

Materials and methods

Oxidative stress experiments for XAS

Oxidative stress experiments for XAS were conducted as described previously (Küpper *et al.*, 2008). *L. digitata* sporophytes (approx. 2–100 cm in size) were collected in the sublittoral at Roscoff (Brittany) and Helgoland (Germany) and kept in tanks with aerated, running seawater between 4–10 °C until use. Oligoguluronate elicitors (GG) were applied at a final concentration of 100 µg ml^{-1}. Typically, experiments were conducted with around 5 g *Laminaria* fresh weight in 100 ml natural seawater, from which both tissue samples of approximately 0.5 g and aliquots of seawater medium of 15 ml were removed directly at the onset of the stress, and then at intervals of 1, 3, 5, 10, 15, 30, 45, 60, 180 minutes, and 24 hours, respectively. *L. digitata* tissue samples were cut out of the phylloid blade and blotted dry on paper tissue. They were immediately fitted into a plexiglass frame and sealed with Capton tape. All samples were immediately frozen in liquid nitrogen. This experiment was

repeated around 15 times with minor variations in sample volume, total algal biomass, and seawater volume.

Preparation of different Laminaria *tissue samples, extracts, and enzymes for XAS*

Isolated cell walls and a polyphenol-enriched fraction were prepared from *L. digitata* sporophytes collected in the sublittoral at Roscoff (Brittany), as described previously (Mabeau and Kloareg, 1987; Connan *et al.*, 2006). The polyphenol-enriched fraction is a methanol/water extract (50:50) of *Laminaria* blades at 40 °C followed by vacuum drying. An industrial alginate sample was obtained from a major alginate manufacturer (DANISCO); the production followed a well-established technique (McHugh, 1987). The other tissue samples were prepared as described before (Küpper *et al.*, 2008), by dissecting, blotting/drying, and sealing in a plexiglass frame sealed with Capton tape, followed by flash-freezing in liquid N_2. The extraction and purification of the native VHPOs from *L. digitata* have been described previously (Colin *et al.*, 2003).

ICP-MS analysis of total iodine bromine

Iodine and bromine contents in freeze-dried *L. digitata* tissue and cell-wall samples were analysed by ICP-MS after pyrohydrolysis separation, as described previously (Schnetger and Muramatsu, 1996; Chai and Muramatsu, 2007). Concentrations obtained are given in ppm (i.e. µg halogen per g of freeze-dried material).

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 (Feiters *et al.*, 2005a). PDB files for the multiple scattering units of the aromatic amino acids were generated using ChemBio3D Ultra.

Neutrophil oxidative burst assay for determining the antioxidant potential of bromide

Blood was withdrawn from healthy volunteers into blood collection tubes containing sodium citrate (Sarstedt). Neutrophils were isolated using density gradient centrifugation and resuspended in Hanks Balanced Salt solution at a density of 1,000,000 cells ml^{-1}. Neutrophils were mixed 1:1 with 20 µM 2',7'-dichlorofluorescein diacetate and incubated (37 °C, 5% CO_2) for 30 minutes prior to seeding into a 96-well plate. Test compounds were added to triplicate wells at a range of concentrations (0.01–100 mM). Oxidative burst was stimulated by addition of 0.1 nM phorbol myristate acetate (Repine *et al.*, 1974). The plate was incubated at 37 °C with fluorescence readings taken every 10 minutes. V_{max} was calculated for each test group over four data points. Percentage activity was calculated by comparing test group V_{max} to control V_{max}.

EPR antioxidant assay

The antioxidant potential of KBr and KI was determined by EPR. Stock solutions of KI and KBr (0.10 M in H_2O) were diluted as required to the final working concentration. Both compounds were tested with the radical-generating systems menadione (in DMSO) and pyrogallol (in H_2O) at a final concentration of 150 µM in the presence of the spin trap, tempone-H (50 µM). Both menadione and pyrogallol undergo auto-oxidation under oxic (atmospheric oxygen) conditions to generate oxygen-centred radicals. The spin-trap chosen is recognized to be selective for oxygen-centred radicals (Meja *et al.*, 2008). Menadione incubation was performed in double-distilled H_2O, and pyrogallol incubation in PBS, with appropriate vehicle controls (i.e. PBS or H_2O only) run as blanks. Incubations were performed at 37 °C with readings taken at 0, 15, 30, 45, and 60 min using a benchtop MS200 X-Band EPR spectrometer (Magnettech, Berlin, Germany) set with the following parameters: microwave frequency 9.30–9.55 GHz; B_0 field 3344 Gauss; sweep width 50 Gauss; sweep time 60 s; modulation amplitude 2.0 Gauss; microwave power 10 mW. Formation of the spin-adduct (4-oxo-tempo) by oxidizing radical species was measured and the amplitude of the first derivative spectra obtained (arbitrary units) was plotted against time following subtraction of appropriate blank measurements. Radical generation was estimated by measuring the area under the resultant linear plot for each individual 60-min incubation, prior to mean and standard error calculations (Graphpad Prism version 5.0).

Results and discussion

Very little is known about the chemical biology and atmospheric chemistry of bromine, despite the accumulating wealth of knowledge for iodine regarding physiology and biochemistry of animal and algal systems and its importance in biogeochemical cycles (Carpenter *et al.*, 2009; Küpper *et al.*, 2011). Brominating oxidants are formed by eosinophils, capable of destroying a wide range of prokaryotic and eukaryotic targets (Weiss *et al.*, 1986) and marine macroalgae are known to emit bromocarbons at high rates (Carpenter and Liss, 2000; Carpenter *et al.*, 2000). The recent discovery that the accumulation of iodide serves the provision of a simple, inorganic antioxidant in the kelp *Laminaria* (Küpper *et al.*, 2008) prompted the current investigation of the chemical speciation and biological significance of bromine in this important model system with a similar, interdisciplinary array of approaches and techniques.

The first objective was to determine bromine levels in various types of *Laminaria* tissues by ICP-MS. Most of the samples studied had previously served for XAS analyses reported below. Overall, the ICP-MS results of bromine levels in *L. digitata* blades and stipes (Table 1; for *Laminaria* anatomy, see Supplementary Fig. S1, available at *JXB* online) were consistent with previous results measured in freeze-dried and chemically fixed tissues using neutron activation analysis (Verhaeghe *et al.*, 2008b). The highest bromine levels were encountered in the holdfast (≈ 2000 ppm) and cortical stipe tissues (≈ 1800–1900 ppm), while the lowest concentrations were found in foliar blade (966 ± 77 ppm) and medullary stipe tissues (654 ± 26 ppm). The highest iodine levels (also measured by ICP-MS), up to more than 57,000 ppm, were observed in outer cortical tissues of *Laminaria* stipes, followed by the inner cortex (≈40,000 ppm) while the lowest tissue concentrations were found in the medulla of stipes (≈1000 ppm). Foliar blade tissues contained close to 5000 ppm (but due to the anatomy of the blade, it was not possible to separate cortical from medullary tissue; it is assumed that the cortex would show much higher iodine levels than the medulla). There was a clear pattern of higher bromine and iodine concentrations in cortical, foliar blade and holdfast tissues as opposed to the thallus medulla, underlining the characteristics of halogen accumulation as a brown algal surface process. Overall, accumulation of iodine was much more pronounced than that of bromine with total concentrations up to over an order of magnitude higher. Considering the much lower concentration of iodine in seawater (0.5 µM in total, with about equal levels of iodide and iodate) in comparison to bromide (0.8 mM), the accumulation

Table 1. Total non-volatile iodine and bromine content of samples of various tissue parts and cell-wall components of *Laminaria digitata*. Determined by ICP-MS. Algal tissue samples were freeze-dried and ground in liquid nitrogen. *n*, technical replicates.

Sample	Iodine concentration (mean ppm)	*n*	Standard deviation (%)	Bromine concentration (mean ppm)	*n*	Standard deviation (%)
Foliar blade tissues from *Laminaria digitata*						
1st whole blade	15505	2	16	714	1	–
2nd whole blade	4834	4	1	966	4	8
Stipe tissues from *Laminaria digitata*						
Whole stipe sections (about 0.4 cm in diameter)	12790	2	6	1465	1	–
1st dissected medullary tissues	1188	4	6	1019	4	6
2nd dissected medullary tissues	1670	3	5	654	3	4
Dissected inner cortex tissues	39708	4	6	1940	2	1
Dissected outer cortex and epidermis tissues	57717	4	16	1794	1	
Holdfast tissues from *Laminaria digitata*						
1st whole holdfast	8057	4	22	2016	4	14
2nd whole holdfast	9715	4	3	1864	4	9
Industrial sodium alginate batch extracted from *Laminaria digitata* (Danisco, Landerneau, France)	20	3	15	12	3	37

factor of iodine from seawater to *Laminaria* tissues (around five orders of magnitude) was much more extreme than for bromine (less than 1 order of magnitude). In contrast, industrial sodium alginate from *L. digitata* contained very low and similar bromine (12 ± 4 ppm) and iodine levels (20 ± 3 ppm).

XAS was used to elucidate the *in vivo* speciation of bromine and to compare it to that of iodine. Representative Br K-edge X-ray absorption near-edge spectra are shown in Supplementary Fig. S2 and Br K-edge extended X-ray absorption fine structure (EXAFS) and corresponding Fourier transforms are shown with their simulations in Figs. 1–3. As described in detail in the supporting information (available at *JXB* online), three types of contributions to the biological Br EXAFS spectra obtained with *Laminaria* thallus samples could be distinguished, which were representative of three different chemical environments, viz. Br^- surrounded by hydrogen-bonding molecules (Fig. 1),

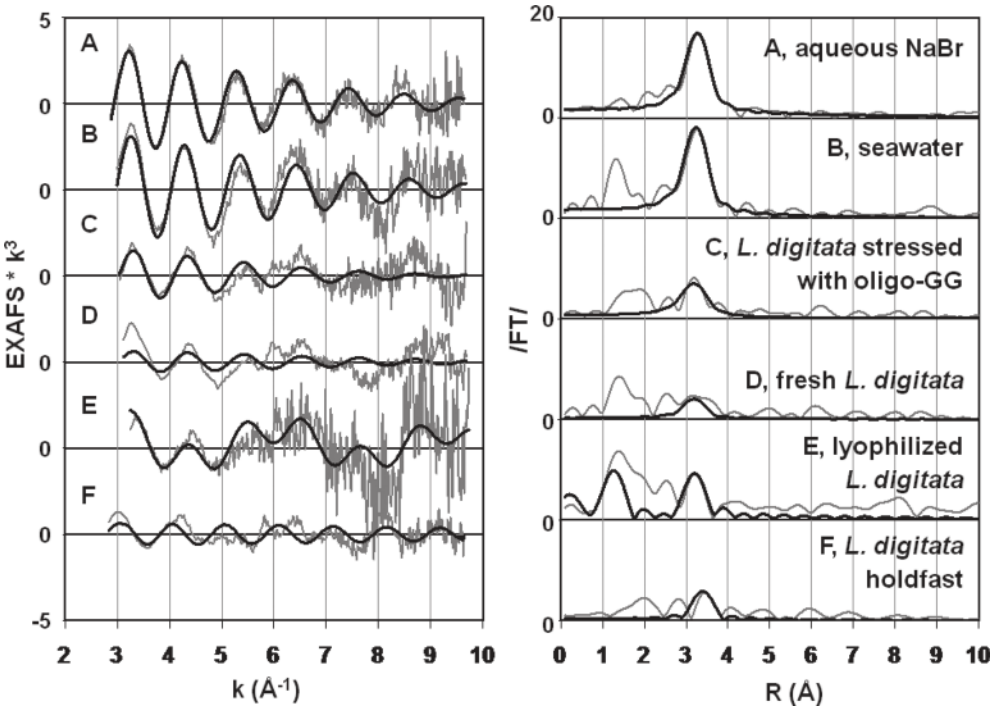

Fig. 1. *Laminaria* tissues versus NaBr solution and seawater. Br K-edge experimental (grey line) and simulated (black line) EXAFS (left) and phase-corrected Fourier-transforms (right): (A) NaBr solution (number and type of atoms @ distance in Å (Debye-Waller factor as $2\sigma^2$ in $Å^2$): 9.5 O @ 3.335 (0.030)), (B) seawater (9.5 O @ 3.300 (0.028)), (C) *L. digitata* stressed with oligo-GG (5.0 O @ 3.262 (0.039)), (D) fresh *L. digitata* blotted (1.8 O @ 3.254 (0.023)), (E) lyophilized *L. digitata* (2.2 H @ 1.283 (0.002) and 2.1 O @ 3.238 (0.006)), and (F) *L. digitata* holdfast (1.7 O @ 3.443 (0.009)). See also Supplementary Table S1.

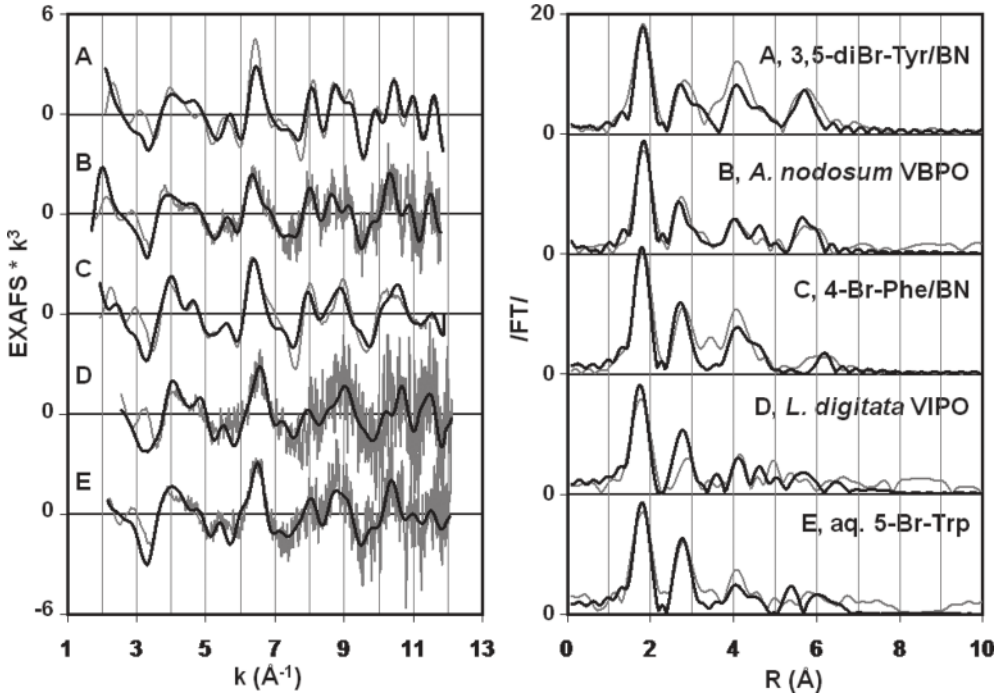

Fig. 2. Brown algal haloperoxidases and brominated amino acids. Br K-edge experimental (grey line) and simulated (black line) EXAFS (left) and phase-corrected Fourier-transforms (right): (A) 3,5-dibromotyrosine/BN (number and type of atoms @ distance in Å (Debye-Waller factor as $2\sigma^2$ in Å^2): 1.0 diBrTyr with C @ 1.882 (0.003)), (B) *A. nodosum* bromoperoxidase (1.0 diBrTyr with C @ 1.906 (0.002)), (C) 4-bromophenylalanine/BN (1.0 phenyl with C @ 1.856 (0.004)), (D) *L. digitata* iodoperoxidase (1.0 indolyl with C @ 1.847 (0.003)), and (E) aqueous 5-bromotryptophan (1.0 indolyl with C @ 1.862 (0.006)). See also Supplementary Table S1.

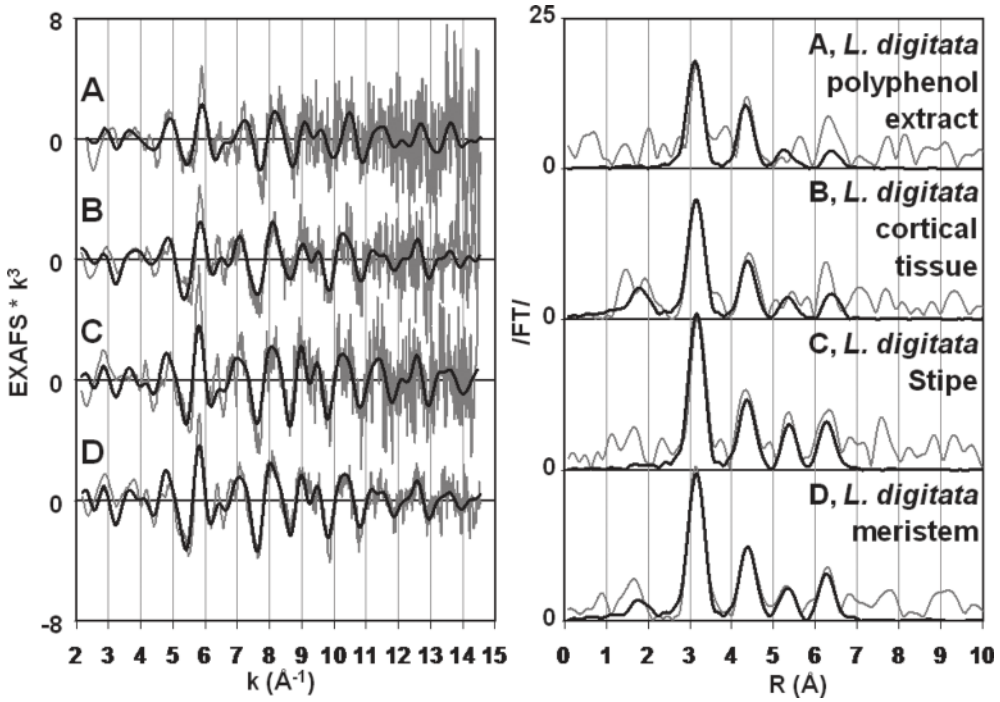

Fig. 3. Different *Laminaria* extracts and tissues. Br K-edge experimental (grey line) and simulated (black line) EXAFS (left) and phase-corrected Fourier-transforms (right): (A) polyphenol-enriched extract (number and type of atoms @ distance in Å (Debye-Waller factor as $2\sigma^2$ in Å^2): 1.4 K @ 3.173 (0.008)), (B) cortical tissue (1.8 K @ 3.203 (0.010) and 0.5 C @ 1.907 (0.008)), (C) stipe (2.0 K @ 3.211 (0.007) and 0.1 C @ 1.864 (0.008)), and (D) meristem (2.4 K @ 3.207 (0.010) and 1.890 (0.007)). See also Supplementary Table S1.

Br covalently incorporated into aromatic moieties (Fig. 2), and Br^- incorporated in solid KCl (Fig. 3), with examples of the corresponding edge X-ray absorption near-edge spectra in Supplementary Fig. S2 (traces A, C/D, and B, respectively). Bromine and iodine K-edge EXAFS and corresponding Fourier transforms are given in Supplementary Figs. S3 and S4, and all iterative refinement parameters of the simulations for Br and I are given in Supplementary Tables S1 and S2, respectively. The XAS results in Fig. 1 clearly show that bromide is the dominant form of bromine in *Laminaria*, and that it is present in a non-covalently bound form, associated with heteroatoms from biomolecules by hydrogen-bonding. Br was found to be predominantly hydrogen-bonded to biomolecules in fresh and lyophilized *Laminaria* (Fig. 1, traces D and E), but the larger number of oxygen atoms found for oligo-GG stress (Fig. 1, trace C) compared to fresh *Laminaria* indicated that a larger fraction of it was hydrated under oligo-GG stress rather than bound to biomolecules in fresh *Laminaria*. This is analogous to the situation reported earlier (Küpper *et al.*, 2008) for iodide in *Laminaria*, and it is in line with the differences observed between the dissolving of iodide by small solvent molecules, resulting in interaction with many solvent atoms, or by larger solvent molecules, where fewer solvent atoms can interact (Tanida and Watanabe, 2000). In contrast, the XAS spectra of freeze-dried *Laminaria* tissues rehydrated with 2 mM H_2O_2 (Supplementary Fig. S3, trace A) could be interpreted by the incorporation of bromide in aromatic substrates both in the rehydrated thallus and in the cell-wall fraction, either directly by the oxidative activation of Br^- by H_2O_2 or by the action of haloperoxidases. An analogous observation was made for iodide in *Laminaria* (Supplementary Fig. S4, trace H; Küpper *et al.*, 2008). Of the *Laminaria* parts, the cell wall was the only one that displayed this spectrum (Supplementary Fig. S3, trace B). While the spectrum of the *Laminaria* holdfast (Fig. 1, trace F) resembled that of whole *Laminaria*, indicating the presence of H-bonded Br^-, those of other *Laminaria* thallus parts (apart from the blade) in Fig. 3, viz. the methanol extract enriched in polyphenols, the cortical tissue, the stipe, and the meristem, appeared to contain bromide in the position of a Cl defect in a KCl crystal. It is possible to interpret this result as due to the presence of free Br^- which co-precipitates with KCl upon cooling of the sample for the XAS measurement, but the concept that Br^- is also associated with solid KCl under physiological conditions in *Laminaria* cannot be excluded. It should be noted that the highest concentrations of K^+ and Cl^- ions in the cortex and medulla of dry weight *Laminaria* (120,000 and 109,000 ppm, respectively (Verhaeghe *et al.*, 2008a), which turn out to be equimolar amounts upon correction for the atomic weight) imply that 22.9 % of the solid material was KCl. The new data are in accordance with the report of bromine localization in *Laminaria* (Verhaeghe *et al.*, 2008a), which suggests that bromine is less diffusible than iodine in stipe tissues and is proportionally more abundant in strong association with the cell walls. By merging these different approaches – microchemical imaging (Verhaeghe *et al.*, 2008b), XAS, ICP-MS, EPR, and whole-blood antioxidant assays – this study sought to provide new hypotheses about bromination processes and the function of bromine in brown algal kelps.

A comparison of the pattern of peaks in the Fourier transform of the Br EXAFS for the native *L. digitata* VHPOs (Fig. 2D for iodoperoxidase, Supplementary Fig. S3C for bromoperoxidase) with a number of brominated amino acids (3-Br-tryptophan, 4-Br-phenylalanine, and 3,5-di-Br-tyrosine in Figs. 2E, 2C, and 2A, respectively) showed evidence for incorporation of Br into the aromatic moiety of the amino acids. This was, however, not in the form of 3,5-dibromo-tyrosine as established earlier for the bromoperoxidase of *Ascophyllum nodosum* by XAS (also included in Fig. 2 as trace B) and mass spectrometry (Feiters *et al.*, 2005b). Simulations of the *Laminaria* haloperoxidase Br XAS with structural models based on 5-Br-tryptophan or 4-bromo-phenylalanine, respectively, confirmed that the Br was attached to an aromatic group, but did not allow unambiguous identification of the type of amino acid (3-bromo-tyrosine, 4-bromophenylalanine, 5-bromotryptophan), except for the exclusion of 3,5-dibromo-tyrosine. The native bromoperoxidase of *A. nodosum* contained Br in the surface tyrosine residues 398 (rarely monobrominated) and 447 (frequently dibrominated) (Feiters *et al.*, 2005b). In fact, the alignment of the different VHPO proteins did not show a strict conservation of tyrosine residues at these two positions, but phenylalanine residues in *L. digitata* VHPOs (Supplementary Fig. S5). Both types of native VHPOs from *Laminaria* were brominated; the enzymes appeared to have been exposed to conditions in the algae or during enzyme extraction where they were brominated. It should be noted that the bromoperoxidase is capable of brominating itself, whereas the iodoperoxidase is unable to react with bromide (Colin *et al.*, 2005).

XAS of iodine in *Laminaria* (Supplementary Fig. S4) shows that it was present as iodide, which is hydrogen-bonded to large biomolecules in fresh *Laminaria*, but partly mobilized as hydrated iodide upon addition of an oligo-GG elicitor, as reported earlier (Küpper *et al.*, 2008), and of H_2O_2. The iodine EXAFS showed no evidence of incorporation in KCl crystals, as found for the bromine. Iodide has been found (Verhaeghe *et al.*, 2008b) to be associated more exclusively than bromide with peripheral tissue, where the potassium and chloride concentrations are relatively low; moreover, the iodide ion would fit even more poorly in the KCl lattice than the bromide ion, due to its larger size.

Consistent with previously published results (Carpenter *et al.*, 2000; Palmer *et al.*, 2005; Thomas *et al.*, 2011), *Laminaria* emitted predominantly brominated halocarbons in the unstressed steady state, while an oxidative burst following GG treatment resulted in strongly increased emissions of iodocarbons (Supplementary Fig. S6). Arguably, the more physiological form of oxidative stress (caused by GG) was met by a mobilization of the iodide antioxidant reservoir, thus providing cell-wall-bound haloperoxidase enzymes both with their preferred substrate and also at a locally higher concentration than bromide, leading to an increased incorporation of iodine into organic molecules, resulting in mono- and multiple iodinations. However, it has to be noted that the total emission rate of iodine via iodocarbons was at least three orders of magnitude lower than the inorganic iodine efflux previously reported (Küpper *et al.*, 1998, 2008; Chance *et al.*, 2009). This efflux was dominated by CH_2I_2, CH_3I, $CHBr_2I$, and C_2H_5I

(Supplementary Fig. S6B). Interestingly, both vanadium iodoperoxidase and vanadium bromoperoxidase genes are upregulated after GG elicitation (Cosse *et al.*, 2009) and *in silico* protein sequence targeting suggested cell-wall localization (Colin *et al.*, 2003, 2005). Collén *et al.* (1996) found that adding H_2O_2 to *Meristiella gelidium* increased production of bromo- and chlorocarbons whereas iodocarbon levels were unaltered. Comparison of exogenous oxidative stress (exogenous H_2O_2) versus a GG-elicited oxidative burst (Supplementary Fig. S6A,B) suggested that in *L. digitata*, simple extracellular addition of H_2O_2 did not adequately mimic the rapid physiological ROS formation plus mobilization of accumulated iodide and volatile halocarbons that occurs during a GG-triggered oxidative burst. Under stress conditions, spontaneous chemical reaction of I^- with H_2O_2 and the activity of constitutive algal haloperoxidases are likely to generate hypoiodous acid (HOI) in equilibrium with I_2. HOI and I_2 are highly reactive and readily react with dissolved organic matter to produce organoiodine compounds (Truesdale *et al.*, 1995; Bichsel and von Gunten, 1999; Carpenter *et al.*, 2005), analogous to bromocarbon synthesis by HOBr/bromoperoxidase (Theiler *et al.*, 1978). The rates of production of iodine- and bromine-containing compounds will depend on the relative concentrations of I^- and Br^-, the specificity of the haloperoxidase enzymes, dissolved organic matter availability, and type. Carbon skeletons could potentially originate from the alga itself (e.g. from the release of free fatty acids), which would then be iodinated and oxidized in the strongly oxidizing conditions prevalent during the oxidative burst (Küpper *et al.*, 2001). Critically for the production of volatile halocarbons, the substantial I^- efflux would shift the balance between Br^- and I^-, increase HOI production relative to HOBr, and swamp the haloperoxidase system with iodide such that iodocarbons dominate the blend of halocarbon volatiles produced. The absence of a bromide efflux during oxidative stress (supporting information) contrasted with the strong iodide efflux observed under the same conditions (Küpper *et al.*, 2008; Chance *et al.*, 2009) due to the predominance of iodide accumulation compared to bromide.

An antioxidant assay based on phorbol myristate acetate-stimulated human blood cells showed that KBr has significant antioxidant activity only at the highest concentration tested, 100 mM (Fig. 4). At all lower concentrations (0.01, 0.1, 1 and 10 mM), no significant anti- or pro-oxidant effect was detected. Remarkably, in the same assay, iodide showed a pro-oxidant effect at 0.01, 0.1, 1, and 10 mM which, however, was attenuated at 10 mM KI by the presence of 1 mM KBr. Only the highest iodide concentration, 100 mM, had a strong antioxidant effect in this assay.

The results of the *in vitro* EPR assay that discriminated between scavenging of superoxide and hydroxyl radicals are shown in Fig 5. KBr showed a trend towards scavenging of superoxide at concentrations >1 mM, reaching statistical significance at 100 mM. KI failed to scavenge superoxide at the same concentrations and showed a trend for pro-oxidant activity. In contrast, both KBr and KI exhibited complex interactions with hydroxyl radicals, with a pro-oxidant effect at moderate concentrations (10–100 μM) being overwhelmed by antioxidant effects at high concentrations (significant at 100 mM). The results show that the reactivity of the inorganic halide with radicals is complex and concentration dependent. High Br^- concentrations (>1 mM) were found to scavenge superoxide whilst equivalent concentrations of I^- tended to exacerbate radical generation. In contrast, both halides increased radical generation at moderate (10–100 μM)

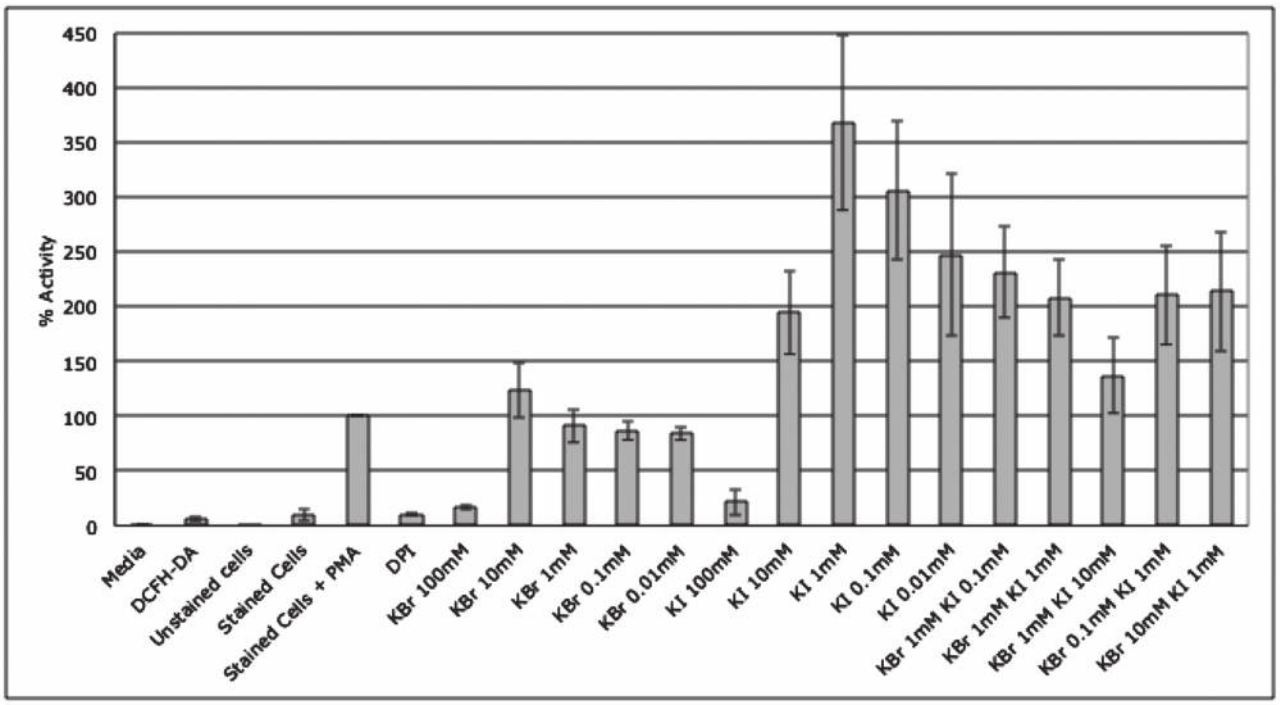

Fig. 4. Neutrophil antioxidant assay at varying concentrations of bromide and iodide.

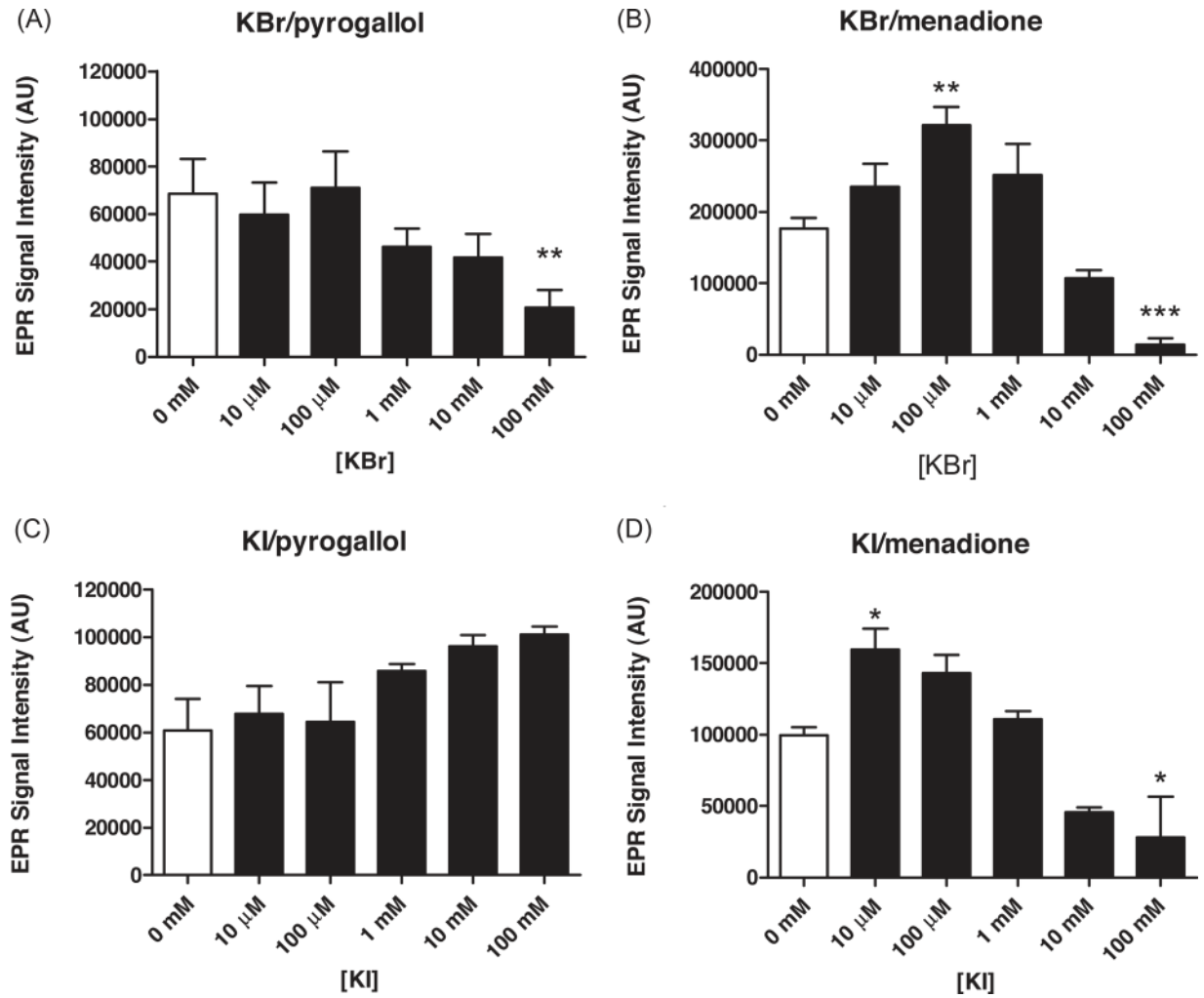

Fig. 5. Electron paramagnetic resonance determination of ROS by spin trapping. Effect of increasing concentrations of KBr (A, B) and KI (C, D) on spin adduct formation in the presence of superoxide (pyrogallol; A, C) and hydroxyl (menadione; B, D) radical generators. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to control (0 mM KI or KBr): Dunnett's post-test after one-way ANOVA.

concentrations and were equally effective at sequestering hydroxyl radicals at concentrations >1 mM. Indeed, similar concentration-dependent pro- and antioxidant behaviour is known for a range of biological antioxidants (Long and Halliwell, 2001). At present, there is no evidence to support a mechanism for the phenomenon in the case of iodide and bromide, but it is tempting to hypothesize that it might (as is the case with some other antioxidants) hinge around the balance between reduction of metal ions to more reactive reduced forms for mediating Fenton chemistry (e.g. Fe^{2+}; pro-oxidant) and radical-scavenging activity (antioxidant).

This study shows that *L. digitata* sporophytes contain bromine as bromide, similarly to the more pronounced accumulation of iodide, but to a lesser degree and also bound to aromatic molecules. The chemical *in vivo* speciation of bromide in *Laminaria* resembles that of iodine – it is overwhelmingly associated with biomolecules via hydrogen bonds. Also similarly to the previously documented case of iodide (Küpper *et al.*, 2008), oxidative stress results in increased hydration. However, and even though in principle the same reaction pathways would apply for both bromide and iodide (such as halide-assisted disproportionation of hydrogen peroxide; Vilter, 1995; Küpper *et al.*, 2008), bromide appears to be less suitable as an antioxidant than iodide, based on both thermodynamic and kinetic considerations regarding the reactions of halides with the oxidants relevant in a living system. Its function is likely not only that of an antioxidant but also, as found in other brown algae not showing a specific iodine concentration, a matter of interacting macromolecules (such as cross-linking in *Fucales*; Potin and Leblanc, 2006), and involvement in different and complementary roles for chemical defence, as also found in red algae (such as disruption of the quorum-sensing systems of bacterial biofilms; Butler and Carter-Franklin, 2004).

However, the antioxidant function of iodide clearly depends on the nature of the oxidant considered, as established by neutrophil oxidative burst and EPR assays. With regard to superoxide and as highlighted by both the neutrophil oxidative burst and EPR assays, iodide was actually pro-oxidant between 0.1–10 mM; only higher concentrations were antioxidant. However, superoxide did not accumulate during oxidative stress in *Laminaria* but was rapidly

converted to hydrogen peroxide by the action of superoxide dismutase (Küpper *et al.*, 2001) and scavenged by bromide (this study; Fig. 5). In contrast, iodide is a very efficient antioxidant against hydrogen peroxide, hydroxyl radicals, and ozone (Küpper *et al.*, 2008), and it is well established that at least hydrogen peroxide (Küpper *et al.*, 2001, 2002) and ozone (Palmer *et al.*, 2005; Küpper *et al.*, 2008) can exist at concentrations physiologically relevant for *Laminaria*. In one-electron oxidations, only iodide – not bromide – reacts exergonically with hydroxyl and superoxide radicals (Luther III, 2011). Among the two-electron reactions, iodide is still by far the most favourable reaction partner for many biologically relevant oxidants (ozone, singlet oxygen, and hydrogen peroxide), even though bromide and chloride show favourable (albeit considerably smaller) ΔG_R values (Luther III, 2011) for the reaction with ozone and hydrogen peroxide (and the EPR data suggest that bromide might have some additional benefits over iodide with regard to superoxide radical). Similarly, the reaction of iodide with ozone, singlet oxygen, and hydrogen peroxide was several orders of magnitude faster than that of bromide or chloride (Table 2). Finally, it should be highlighted that iodide is locally the predominant halide species and could be the preferred substrate of cell-wall-localized vanadium haloperoxidases over bromide and chloride (Verhaeghe *et al.*, 2008a). Haloperoxidases also considerably accelerate the halide-assisted disproportionation of hydrogen peroxide (Vilter, 1995; Küpper *et al.*, 2008).

The EPR results highlight the importance of having the correct blend of antioxidants present in the correct compartment in order to ensure beneficial as opposed to detrimental or neutral effects. For example, high concentrations of Br^-, but not I^-, could represent a useful antioxidant in defence against superoxide (e.g. oxidative burst), whilst high (but not moderate) concentrations of both Br^- and I^- could protect against hydroxyl radical (e.g. derived from hydrogen peroxide via Fenton chemistry). From an evolutionary perspective, therefore, it would prove advantageous to maintain large stores of halides in inactive (caged) forms (i.e. bound to organic entities) and to release them in high concentrations in response to stress. A combined assault with Br^- and I^- would have the added benefit of targeting different ROS species, thus providing comprehensive protection.

Overall, in *Laminaria*, bromide compares with iodide as follows. (i) There is no strong accumulation of bromide from

Table 2. Kinetics of oxygen species with halides and other reductants.

Compound	k_{12} (M^{-1} s^{-1})	Source and notes
O_3 reactions with:		
I^-	1.2×10^9	Liu *et al.* (2001)
Br^-	2.48×10^2	Liu *et al.* (2001)
Cl^-	$<3\times10^{-3}$	Hoigné et al. (1985)
Ascorbate	4.8×10^7	Kanofsky and Sima (1995)
Glutathione	2.5×10^6	Kanofsky and Sima (1995)
Singlet oxygen ($^1O_2$) reactions with:		
I^-	1×10^8	Rosenthal (1976; aprotic solvents)
	8.7×10^5	Wilkinson *et al.* (1995, p 896; pH ~7)
Br^-	1.0×10^3	Wilkinson *et al.* (1995, p 895; in D_2O)
Cl^-	1.0×10^3	Wilkinson *et al.* (1995, p 895; in D_2O)
Ascorbate	8.3×10^6	Wilkinson *et al.* (1995, p 904; pH 6.8)
Glutathione	2.4×10^6	Wilkinson *et al.* (1995, p 883; in D_2O, 310 K, *p*D 7.4)
OH radical (·OH) reactions with:		
I^-	1.2×10^{10}	Buxton *et al.* (1988, p 527)
Ascorbate	1.1×10^{10}	Buxton *et al.* (1988, p 700)
Glutathione	1.3×10^{10}	Buxton *et al.* (1988, p 723; pH 5.5)
Dimethyl sulphoniopropionate	3×10^8	Sunda *et al.* (2002)
Dimethyl sulphide	1.9×10^{10}	Buxton *et al.* (1988)
Dimethyl sulphoxide	6.6×10^9	Buxton *et al.* (1988)
Superoxide (O_2^-) reactions with:		
I_3^-	1×10^8	Bielski *et al.* (1985, p 1063; no data available for I^-)
Ascorbate	2.7×10^5	Bielski *et al.* (1985, p 1069; pH 7.4)
Glutathione	2.4×10^5	Bielski *et al.* (1985, p 1075; pH 7.8)
Hydrogen peroxide (H_2O_2) reactions with:		
I^-	0.69	Mohammed and Liebhafsky (1934)
Br^-	2.3×10^{-5}	Mohammed and Liebhafsky (1934)
Cl^-	1.1×10^{-7}	Mohammed and Liebhafsky (1934)
Ascorbate	2	Polle and Junkermann (1996)
Glutathione	2–20	D'Autréaux and Toledano (2007)
Glutathione peroxidase	6×10^7	Flohé *et al.* (1972)

Table 3. Comparison of the key features of iodine and bromine metabolism in *Laminaria*.

Feature	Iodine	Bromine
Accumulation factor (from seawater to *Laminaria*)	10^4–10^5	1–10^1
Efflux upon oxidative stress	Yes	Not detected
Halocarbon emission	Iodinated halocarbons are emitted mainly after the oxidative burst	Bromocarbons are emitted at high rates under unstressed, steady-state conditions
Detection of oxidized species upon oxidative stress	No	No
Antioxidant effect in whole-blood assay	Yes	Partial

seawater, and no detectable bromide efflux upon oxidative stress. (ii) An oxidative burst results in a shift to increased iodocarbon emissions (this study; Palmer *et al.*, 2005; Thomas *et al.*, 2011). (iii) The reaction of bromide with most of the biologically relevant oxidants is not favourable, neither in a thermodynamic nor in a kinetic sense. Therefore, the results suggest that, while the extracellular antioxidant defence of *Laminaria* is primarily based on iodide, iodide and bromide may complement each other in the face of different oxidants such as superoxide and that the role of bromine and brominated compounds in macroalgae is more complex than previously thought (Table 3).

This work constitutes the first study about the physiology of bromide as a potentially comparable antioxidant. The results show that bromide complements iodide as an inorganic, extracellular antioxidant in *Laminaria*, in particular against superoxide, but also that it is less effective against most other biologically relevant oxidants and that its function is more diverse than that of iodide. It is proposed to call the preferential accumulation and targeted release of iodide upon oxidative stress, in comparison to bromide, the 'iodide switch'. This work suggests that the accumulation of iodide and the iodide switch are central and unique features of the evolution of morphologically complex, large brown algal kelps such as *Laminaria*. The evolutionary origin of this unique antioxidant system remains one of the most intriguing research questions in this context.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. Scheme of *Laminaria digitata* showing the different parts of the thallus

Supplementary Fig. S2. Br K-edge XANES and EXAFS of four representative samples: seawater and *L. digitata* stipe, cell wall, and lyophilized *L. digitata* rehydrated with H_2O_2

Supplementary Fig. S3. Br K-edge EXAFS and phase-corrected Fourier-transforms of lyophilized *L. digitata* rehydrated with H_2O_2, cell wall, *L. digitata* native bromoperoxidase, and inactive *Escherichia coli*-expressed iodoperoxidase with added Br

Supplementary Fig. S4. I K-edge EXAFS and phase-corrected Fourier-transforms of fresh *L. digitata*, *L. digitata* stressed with oligo-GG or H_2O_2, and lyophilized and rehydrated *L. digitata*

Supplementary Fig. S5. Partial amino-acid alignment of vanadate-dependent haloperoxidases

Supplementary Fig. S6. Release of volatile low-molecular-weight halocarbons from *L. digitata* plantlets

Supplementary Table S1. Parameters resulting from iterative refinement of simulations of the *Laminaria* bromine-edge EXAFS

Supplementary Table S2. Parameters resulting from iterative refinement of simulations of the *Laminaria* iodine-edge EXAFS

Acknowledgements

The authors are grateful to Dieter Hanelt and the staff at AWI-Biologische Anstalt Helgoland for providing *L. digitata* for the XAS experiments in this study. Funding from the UK Natural Environment Research Council (NERC) through the SOLAS and Oceans 2025 (WP4.5) programmes to LJC and to FCK, the Deutsche Forschungsgemeinschaft (Germany) to PMHK, the French National Program 'Toxicologie Nucléaire Environnementale' to PP, EV, and CL, the National Science Foundation and the National Oceanic & Atmospheric Administration to GWL, 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. FCK would also like to extend special thanks to Alison Butler (University of California, Santa Barbara) for her support and useful discussions. 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. GM was supported by NERC Advanced Fellowship GT5/98/8/MS. Thanks also to the EU-PARFORCE (New Particle Formation and Fate in the Coastal Environment) for allowing participation in their Mace Head campaign. The authors would also like to thank Charlie Bavington for dispensing JR from the duties of her employment at Glycomar (Oban) to allow participation in this study.

References

Ar Gall E, Küpper FC, Kloareg B. 2004. A survey of iodine contents in *Laminaria digitata*. *Botanica Marina* **47,** 30–37.

Balard AJ. 1826. Sur une substance particulière contenue dans l'eau de la mer. *Annales de Chimie et de Physique (Paris)* **32,** 337–384.

Baldauf SL. 2003. The deep roots of eukaryotes. *Science* **300,** 1703–1706.

Bhattacharya D, Stickel SK, Sogin ML. 1991. Molecular phylogenetic analysis of actin genic regions from *Achlya bisexualis* (Oomycota) and *Costaria costata* (Chromophyta). *Journal of Molecular Evolution* **33,** 525–536.

Bichsel Y, von Gunten U. 1999. Oxidation of iodide and hypoiodous acid in the disinfection of natural waters. *Environmental Science and Technology* **33,** 4040–4045.

Bielski BHJ, Cabelli DE, Arudi RL, Ross AB. 1985. Reactivity of HO_2/O_2^- radicals in aqueous solution. *Journal of Physical and Chemical Reference Data* **14,** 1041–1100.

Butler A, Carter-Franklin JN. 2004. The role of vanadium bromoperoxidase in the biosynthesis of halogenated marine natural products. *Natural Product Reports* **21,** 180–188.

Buxton GV, Greenstock CL, Helman WP, Ross AB. 1988. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals in aqueous solution. *Journal of Physical and Chemical Reference Data* **17,** 513–886.

Carpenter LJ, Hopkins JR, Jones CE, Lewis AC, Parthipan R, Wevill DJ, Poissant L, Pilote M, Constant P. 2005. Abiotic source of CH_2I_2 and other reactive organic halogens in the sub-Arctic atmosphere? *Environmental Science and Technology* **39,** 8812–8816.

Carpenter LJ, Jones CE, Dunk RM, Hornsby KE, Woeltjen J. 2009. Air-sea fluxes of biogenic bromine from the tropical and North Atlantic Ocean. *Atmospheric Chemistry and Physics* **9,** 1805–1816.

Carpenter LJ, Liss PS. 2000. On temperate sources of bromoform and other reactive organic bromine gases. *Journal of Geophysical Research – Atmospheres* **105,** 20539–20547.

Carpenter LJ, Malin G, Küpper FC, Liss PS. 2000. Novel biogenic iodine-containing trihalomethanes and other short-lived halocarbons in the coastal East Atlantic. *Global Biogeochemical Cycles* **14,** 1191–1204.

Chai JY, Muramatsu Y. 2007. Determination of bromine and iodine in twenty-three geochemical reference materials by ICP-MS. *Geostandards and Geoanalytical Research* **31,** 143–150.

Chance R, Baker AR, Küpper FC, Hughes C, Kloareg B, Malin G. 2009. Release and transformations of inorganic iodine by marine macroalgae. *Estuarine Coastal and Shelf Science* **82,** 406–414.

Cock JM, Sterck L, Rouzé P, *et al.* 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in the brown algae. *Nature* **465,** 617–621.

Colin C, Leblanc C, Michel G, Wagner E, Leize-Wagner E, van Dorsselaer A, Potin P. 2005. Vanadium-dependent iodoperoxidases in *Laminaria digitata*, a novel biochemical function diverging from brown algal bromoperoxidases. *Journal of Biological Inorganic Chemistry* **10,** 156–166.

Colin C, Leblanc C, Wagner E, Delage L, Leize-Wagner E, Van Dorsselaer A, Kloareg B, Potin P. 2003. The brown algal kelp *Laminaria digitata* features distinct bromoperoxidase and iodoperoxidase activities. *Journal of Biological Chemistry* **278,** 23545–23552.

Collén J, Del Rio MJ, Garcia-Reina G, Pedersén M. 1996. Photosynthetic H_2O_2 production by *Ulva rigida*. *Planta* **196,** 225–239.

Connan S, Delisle F, Deslandes E, Gall EA. 2006. Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. *Botanica Marina* **49,** 39–46.

Cosse A, Potin P, Leblanc C. 2009. Patterns of gene expression induced by oligoguluronates reveal conserved and environment-specific molecular defense responses in the brown alga *Laminaria digitata*. *New Phytologist* **182,** 239–250.

D'Autréaux B, Toledano MB. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nature Reviews Molecular Cell Biology* **8,** 813–824.

Feiters MC, Küpper FC, Meyer-Klaucke W. 2005a. X-ray absorption spectroscopic studies on model compounds for biological iodine and bromine. *Journal of Synchrotron Radiation* **12,** 85–93.

Feiters MC, Leblanc C, Küpper FC, Meyer-Klaucke W, Michel G, Potin P. 2005b. Bromine is an endogenous component of a vanadium bromoperoxidase. *Journal of the American Chemical Society* **127,** 15340–15341.

Flohe L, Loschen G, Günzler WA, Eichele E. 1972. Glutathione peroxidase, V. The kinetic mechanism. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **353,** 987–999.

Gay-Lussac L-J. 1813. Sur un nouvel acide formé avec la substance découverte par M. Courtois. *Annales de Chimie (Paris)* **88,** 311–318.

Hoigné J, Bader H, Haag WR, Staehelin J. 1985. Rate constants of reactions of ozone with organic and inorganic compounds in water – III. Inorganic compounds and radicals. *Water Research* **19,** 993–1004.

Kanofsky JR, Sima PD. 1995. Reactive absorption of ozone by aqueous biomolecule solutions – implications for the role of sulfhydryl compounds as targets for ozone.. *Archives of Biochemistry and Biophysics* **315,** 52–62.

Küpper FC, Carpenter LJ, McFiggans GB, *et al.* 2008. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proceedings of the National Academy of Sciences, USA* **105,** 6954–6958.

Küpper FC, Feiters MC, Olofsson B, *et al.* 2011. Commemorating two centuries of iodine research: an interdisciplinary overview of current research. *Angewandte Chemie – International Edition* **50,** 11598 – 11620.

Küpper FC, Gaquerel E, Boneberg E-M, Morath S, Salaün J-P, Potin P. 2006. Early events in the perception of lipopolysaccharides in the brown alga *Laminaria digitata* include an oxidative burst and activation of fatty acid oxidation cascades. *Journal of Experimental Botany* **57,** 1991–1999.

Küpper FC, Gaquerel E, Cosse A, Adas F, Peters AF, Müller DG, Kloareg B, Salaün JP, Potin P. 2009. Free fatty acids and methyl jasmonate trigger defense reactions in *Laminaria digitata*. *Plant and Cell Physiology* **50,** 789–800.

Küpper FC, Kloareg B, Guern J, Potin P. 2001. Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiology* **125,** 278–291.

Küpper FC, Müller DG, Peters AF, Kloareg B, Potin P. 2002. Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of the sporophytes of *Laminariales*. *Journal of Chemical Ecology* **28,** 2057–2081.

Küpper FC, Schweigert N, Ar Gall E, Legendre JM, Vilter H, Kloareg B. 1998. Iodine uptake in *Laminariales* involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* **207,** 163–171.

La Barre S, Potin P, Leblanc C, Delage L. 2010. The halogenated metabolism of brown algae (Phaeophyta), its biological importance and its environmental significance. *Marine Drugs* **8,** 988–1010.

Liu Q, Schurter LM, Muller CE, Aloisio S, Francisco JS, Margerum DW. 2001. Kinetics and mechanisms of aqueous ozone reactions with bromide, sulfite, hydrogen sulfite, iodide, and nitrite ions. *Inorganic Chemistry* **40,** 4436–4442.

Long LH, Halliwell B. 2001. Antioxidant and prooxidant abilities of foods and beverages. *Flavonoids and Other Polyphenols* **335,** 181–190.

Luther III GW. 2011. Thermodynamic redox calculations for one and two electron transfer steps: implications for halide oxidation and halogen environmental cycling. In: P Tratnyek, T Grundl, SB Haderlein, eds, *Aquatic redox chemistry* , vol. 1071. Washington, DC: American Chemical Society, pp 15–35.

Mabeau S, Kloareg B. 1987. Isolation and analysis of the cell walls of brown algae: *Fucus spiralis*, *F. ceranoides, F. vesiculosus, F. serratus, Bifurcaria bifurcata* and *Laminaria digitata*. *Journal of Experimental Botany* **38,** 1573–1580.

McFiggans GB, Coe H, Burgess R, *et al.* 2004. Direct evidence for coastal iodine particles from *Laminaria macroalgae* – linkage to emissions of molecular iodine. *Atmospheric Chemistry and Physics* **4,** 701–713.

McHugh DJ. 1987. Production, properties and uses of alginates. In: DJ McHugh, ed, *Production and utilization of products from commercial seaweeds* . Rome: FAO, pp 58–115.

Meja KK, Rajendrasozhan S, Adenuga D, *et al.* 2008. Curcumin restores corticosteroid function in monocytes exposed to oxidants by maintaining HDAC2. *American Journal of Respiratory Cell and Molecular Biology* **39,** 312–323.

Mohammed A, Liebhafsky HA. 1934. The kinetics of the reduction of hydrogen peroxide by the halides. *Journal of the American Chemical Society* **56,** 1680–1685.

Palmer CJ, Anders TL, Carpenter LJ, Küpper FC, McFiggans GB. 2005. Iodine and halocarbon response of *Laminaria digitata* to oxidative stress and links to atmospheric new particle production. *Environmental Chemistry* **2,** 282–290.

Polle A, Junkermann W. 1996. Inhibition of apoplastic and symplastic peroxidase activity from Norway spruce by the photooxidant hydroxymethyl hydroperoxide. *Plant Physiology* **104,** 617–623.

Potin P, Leblanc C. 2006. Phenolic-based adhesives of marine brown algae. In: AM Smith, JA Callow, eds, *Biological adhesives* . Berlin and Heidelberg: Springer Verlag, pp 105–124.

Repine JE, White JG, Clawson CC, Holmes BM. 1974. Effects of phorbol myristate acetate on metabolism and ultrastructure of neutrophils in chronic granulomatous disease. *Journal of Clinical Investigation* **54,** 83–90.

Rosenthal I, Frimer, A. 1976. The quenching effect of iodide ion on singlet oxygen. *Photochemistry and Photobiology* **23,** 209–211.

Saenko GN, Kravtsova YY, Ivanenko VV, Sheludko SI. 1978. Concentration of iodine and bromine by plants in the seas of Japan and Okhotsk. *Marine Biology* **47,** 243–250.

Schnetger B, Muramatsu Y. 1996. Determination of halogens, with special reference to, iodine, in geological and biological samples using pyrohydrolysis for preparation and inductively coupled plasma mass spectrometry and ion chromatography for measurement. *Analyst* **121,** 1627–1631.

Strange RW, Feiters MC. 2008. Biological X-ray absorption spectroscopy (BioXAS): a valuable tool for the study of trace elements in the life sciences. *Current Opinion in Structural Biology* **18,** 1–8.

Sunda W, Kieber DJ, Kiene RP, Huntsman S. 2002. An antioxidant function for DMSP and DMS in marine algae. *Nature* **418,** 317–320.

Tanida T, Watanabe I. 2000. Dependence of EXAFS (Extended X-Ray Absorption Fine Structure) parameters of iodide anions in various solvents upon a solvent parameter. *Bulletin of the Chemical Society of Japan* **73,** 2747–2752.

Theiler R, Cook JC, Hager LP, Studa JF. 1978. Halohydrocarbon synthesis by bromoperoxidase. *Science* **202,** 1094–1096.

Thomas F, Cosse A, Goulitquer S, Raimund S, Morin P, Valero M, Leblanc C, Potin P. 2011. Waterborne signaling primes the expression of elicitor-induced genes and buffers the oxidative responses in the brown alga *Laminaria digitata*. *PLOS One* **6,** e21475.

Truesdale VW, Luther GW, Canosamas C. 1995. Molecular-iodine reduction in seawater – an improved rate-equation considering organic compounds. *Marine Chemistry* **48,** 143–150.

Venturi S, Venturi M. 1999. Iodide, thyroid and stomach carcinogenesis: evolutionary story of a primitive antioxidant? *European Journal of Endocrinology* **140,** 371–372.

Verhaeghe E, Buisson D, Zekri E, Leblanc C, Potin P, Ambroise Y. 2008a. A colorimetric assay for steady-state analyses of iodo- and bromoperoxidase activities. *Analytical Biochemistry* **379,** 60–65.

Verhaeghe EF, Fraysse A, Guerquin-Kern J-L, Wu T-D, Devès G, Mioskowski C, Leblanc C, Ortega R, Ambroise Y, Potin P. 2008b. Microchemical imaging of iodine distribution in the brown alga *Laminaria digitata* suggests a new mechanism for its accumulation. *Journal of Biological Inorganic Chemistry* **13,** 257–269.

Vilter H. 1995. Vanadium-dependent haloperoxidases In: H Sigel, A Sigel, eds, *Vanadium and its role in life* , vol. **31**. New York: Marcel Dekker, pp 325–362.

Weiss SJ, Test ST, Eckmann CM, Roos D, Regiani S. 1986. Brominating oxidants generated by human eosinophils. *Science* **234,** 200–203.

Wilkinson F, Helman WP, Ross AB. 1995. Rate constants for the decay and reactions of the lowest electronically excited singlet state of molecular oxygen in solution. An expanded and revised compilation.. *Journal of Physical and Chemical Reference Data* **24,** 663–1023.

Wojtaszek P. 1997. Oxidative burst: an early plant response to pathogen infection. *The Biochemical Journal* **322,** 681–692.

Zambounis A, Gaquerel E, Strittmatter M, Potin P, Küpper FC. 2012. Prostaglandin A_2 triggers a strong oxidative burst in *Laminaria*: a novel defense inducer in brown algae? *Algae* **27,** 1–12.