Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases. Biochimie

ARTICLE *in* BIOCHIMIE · DECEMBER 2006

Impact Factor: 2.96 · DOI: 10.1016/j.biochi.2006.09.001 · Source: PubMed

14 AUTHORS, INCLUDING:

Ludovic Delage
French National Centre for Scientific Resea…
36 PUBLICATIONS **1,027** CITATIONS
SEE PROFILE

Stéphane La Barre
Station Biologique de Roscoff
49 PUBLICATIONS **765** CITATIONS
SEE PROFILE

David Amouroux
French National Centre for Scientific Resea…
212 PUBLICATIONS **3,912** CITATIONS
SEE PROFILE

Philippe Potin
French National Centre for Scientific Resea…
95 PUBLICATIONS **2,640** CITATIONS
SEE PROFILE

Available online at www.sciencedirect.com
ScienceDirect
Biochimie ■■ (2006) ■■■
BIOCHIMIE
www.elsevier.com/locate/biochi

Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases

Catherine Leblanc[a,*], Carole Colin[a], Audrey Cosse[a], Ludovic Delage[a], Stéphane La Barre[a], Pascal Morin[b], Bruno Fiévet[c], Claire Voiseux[c], Yves Ambroise[d], Elodie Verhaeghe[d], David Amouroux[e], Olivier Donard[e], Emmanuel Tessier[e], Philippe Potin[a]

[a] *Centre national de la recherche scientifique, université Pierre et Marie Curie-Paris-VI, laboratoire international Associé-Dispersal and Adaptation in Marine Species, unité mixte de recherche 7139, 29682 Roscoff cedex, France*
[b] *Unité mixte de recherche 7144, station biologique, 29682 Roscoff cedex, France*
[c] *IRSN/DEI/SECRE, laboratoire de radioécologie de Cherbourg-Octeville, rue Max Pol Fouchet, BP 10, 50130 Cherbourg-Octeville, France*
[d] *CEA Saclay, DSV-DBJC-SMMCB, bâtiment 547, 91191 Gif-sur-Yvette, France*
[e] *UMR 5034, LCABIE, CNRS, Helioparc, 2, avenue du Président-Angot, 64053 Pau, France*

Received 20 January 2006; accepted 1 September 2006

Abstract

Brown algal kelp species are the most efficient iodine accumulators among all living systems, with an average content of 1.0% of dry weight in *Laminaria digitata*, representing a ca. 30,000-fold accumulation of this element from seawater. Like other marine macroalgae, kelps are known to emit volatile short-lived organo-iodines, and molecular iodine which are believed to be a main vector of the iodine biogeochemical cycle as well as having a significant impact on atmospheric chemistry. Therefore, radioactive iodine can potentially accumulate in seaweeds and can participate in the biogeochemical cycling of iodine, thereby impacting human health. From a radioecological viewpoint, iodine-129 (^{129}I, half-life of 1.6×10^7 years) is one of the most persistent radionuclide released from nuclear facilities into the environment. In this context, the speciation of iodine by seaweeds is of special importance and there is a need to further understand the mechanisms of iodine uptake and emission by kelps. Recent results on the physiological role and biochemistry of the vanadium haloperoxidases of brown algae emphasize the importance of these enzymes in the control of these processes.

Keywords: Iodine; Volatile halogenated organic compound; Vanadium haloperoxidase; Iodination

1. Introduction

The biogeochemical cycling of iodine has been recently reinvestigated in the light of the importance of this element for human health, hydrologic processes and radioecology [1,2]. It is generally assumed that iodinated compounds are exclusively of biogenic origin since no significant anthropogenic sources are known [3]. Low levels of iodine are found in soils, rivers, lakes and terrestrial plants. By contrast, oceans are the major source of iodine in the geochemical cycle with average concentrations of around 58 μg l^{-1} iodine in seawater. The global cycle of iodine involves intense exchanges in the marine boundary layer (MBL) in which iodine is transferred from the oceans to the atmosphere (Fig. 1). This process occurs primarily by iodovolatilization, i.e. the direct emission of molecular iodine (I_2) and of volatile halogenated organic compounds (VHOC), from the open oceans (mostly via phytoplankton) and coastal areas (mostly via macrophytic algae). These compounds are thus considered as a major carrier of gas phase iodine from the ocean to atmosphere, which in turn supplies iodine in precipitation to marine and terrestrial environments [4,5]. Compared with marine ecosystems, much less

Abbreviations: VHOC, volatile halogenated organic compound; MBL, marine boundary layer; HPO, haloperoxidase; vHPO, vanadium-dependent HPO; vCPO, vanadium-dependent chloroperoxidase; vBPO, vanadium-dependent bromoperoxidase; vIPO, vanadium-dependent iodoperoxidase; MS, mass spectrometry.

* Corresponding author.
E-mail address: leblanc@sb-roscoff.fr (C. Leblanc).

0300-9084/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2006.09.001

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

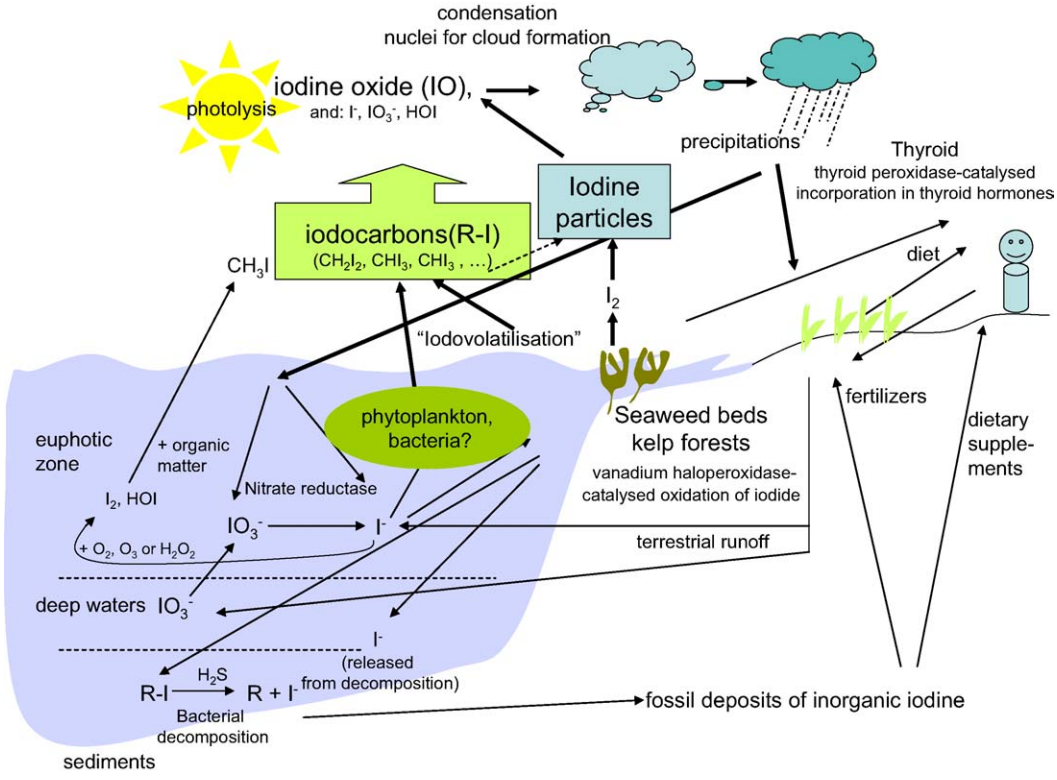

Fig. 1. The global cycle of iodine mainly involves gas exchanges in the MBL with the atmosphere. The current state of knowledge indicates that coastal nucleation events are driven by biogenic emissions that undergo rapid chemical reactions to produce condensable iodine oxides leading to nucleation and growth of new particles. The primary source of the condensable iodine vapors is thought to be molecular iodine (I_2). Photolysis or other processes lead to the formation of highly reactive $I^{\bullet}$ radicals and iodine oxides (IO), impacting the ozone layer. Iodine is deposited on land or back into the sea by rain-falls or aerosols. The limited availability of inorganic iodine on land conditions its incorporation in thyroid hormones by thyroperoxidase, leading eventually to some disorders.

information is available about iodine volatilization from terrestrial environments, although it was recently reported that paddy fields [6] and soil bacteria [7] contribute significantly to iodine emission.

Iodine undergoes a complex chemistry in the atmosphere. Both I_2 and iodocarbons are broken down by photolysis [8] or other processes leading to the formation of highly reactive $I^{\bullet}$ radicals, thereby significantly influencing ozone cycling, especially in the polar lower stratosphere [9]. A variety of short and long lived iodine oxidized species are formed, some as aerosol particles which eventually initiate cloud condensation and subsequent iodine fallout redistribution. On land, this input represents an important source of iodine in the human diet. This update on our knowledge of iodine biogeochemical cycle has been recently reviewed [10,11], and indeed considered as a new research front in a dedicated issue of *Environmental Chemistry* [12], because of the climatic incidences of the phenomenon.

Since the discovery of iodine in kelps as a new chemical element by Courtois in 1811 [13], these brown algae have been a major source of this element. Hence, *Laminaria* species are the strongest iodine accumulators among all living systems, with an average content of 1.0% of dry weight in *Laminaria digitata*, representing an accumulation of this element up to 10^4 times compared to seawater concentration [14]. A particular class of peroxidases, the vanadium-dependent haloperoxidases, was proposed to facilitate iodine transfers across biological membranes and the halogenation of organo-iodine in macroalgae [15,16].

A combination of physiological, biochemical, structural and chemical approaches should allow to confirm the basic mechanism of iodide oxidation into hypoiodous acid and molecular iodine. This mechanism requires constitutive apoplastic levels of hydrogen peroxide of ca. 50 μM, which can be either maintained by active photosynthesis or in response to various stresses. Mimicking such stresses by specific inducers such as oligomers of alginate [17,18], the main kelp cell wall polysaccharide, is of great advantage in deciphering the nature and the regulation of these processes [19]. Then, in combination with a structural characterization of the key enzymes, such as haloperoxidases specific for iodide oxidation, and with an increased knowledge about iodine chemistry in biological solutions, these studies should provide new insights in the context of radioactive and non-radioactive iodine cycling in the marine environment.

2. Speciation of iodine, seaweeds and ocean/atmosphere exchanges

In seawater, dissolved inorganic iodine (ca. 0.5 μM) is known to be mainly present as iodide (I^-) and iodate (IO_3^-) ions in various proportions [20]. In the English Channel, iodide and iodate concentrations are routinely measured at values of

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

ca. 0.35 and 0.25 μM, respectively, with insignificant seasonal fluctuations (Fievet et al., unpublished data).

Organic iodine compounds contribute, in a lesser extent, to the global iodine budget in seawater. In open oceans, methyl iodide (CH_3I) appears to be the dominant iodine organic form, with a mean concentration in the picomolar range [5]. Other various alkyl-iodide compounds, such as ethyl-, propyl-iodide, chloroiodomethane (CH_2ClI), bromoiodomethane (CH_2BrI) and diodomethane (CH_2I_2) also occur in seawater and atmosphere [10,21]. Due to the high chemical reactivity of these compounds in seawater, the different mechanisms involved in volatile iodocarbons production still remain unclear. The abiotic production of CH_3I has been reported via aqueous photochemistry [22,23] and more recently through chemical oxidation processes, involving organic matter in sediments and soils [24]. However, the biogenic production of mono- and polyhalogenated hydrocarbons by both phytoplankton and marine macrophytes has been extensively investigated through field and laboratory experiments [25–34]. In micro- and macroalgae, VHOC production is known to involve halide methyl transferases [35–37] and haloperoxydases, respectively [38].

In the iodine biogeochemical cycle, large uncertainties still remain in assessing global emission fluxes of volatile iodine compounds at the sea-atmosphere interface. First reasonable estimates of marine CH_3I production from phytoplankton range from 1 to 3 × 10^9 mol $years^{-1}$ [5,39,40], whereas macroalgal production of various iodocarbons (i.e. CH_3I, CH_2ClI, CH_2BrI, CH_2I_2) has been found to be two orders of magnitude lower [10]. In other reports, the global oceanic emissions of CH_2I_2 were of similar magnitude (i.e. from 3 to 6 × 10^7 mol $years^{-1}$) and comparable to those of CH_3I [41,42]. Although microalgae are likely to produce polyhalogenated compounds [33], CH_2I_2 is mainly released from macroalgae in coastal areas [43–45] and is even thought to be the main iodine input into the MBL from the shore biota [46].

In coastal environment, brown macroalgae, and especially kelps, are then considered as major contributors to the flux of iodine in the MBL, by iodovolatilization, as reported by early investigators [47–49] or the incorporation of iodine in VHOCs [10,50]. The formation of iodocarbons by kelps was re-investigated in the light of its relationships with oxidative stress [51] and recently of its possible correlation with iodine particle emissions to the atmosphere [19]. This latter study supports the hypothesis that biogenic molecular iodine is a major source of coastal particle formation. Therefore, iodocarbon emissions would contribute to the production of iodine oxides in the atmosphere by processes that are distinct from iodine particle formation. Even if the global significance of volatile iodinated compounds production may be limited, since coastal areas only represent ~0.5% of the total ocean surface [52], the potential importance of iodine emissions from kelp beds for the provision of condensation nuclei for coastal cloud formation was recently highlighted (Fig. 1) [19,53,54]. These new aerosol nanometer-range particles are believed to result from polymerization and condensation of iodine monoxide (IO), the reaction products of iodine with ozone, into higher oxides (OIO, I_2O_5, I_2O_4,…) ([53], for review [11]). The redox state and speciation of iodine in aerosol is thought to play a key role in regulating ozone destruction processes [55,56]. Large fractions of I^-, IO_3^- and organic iodine have been measured in small particle aerosols [57], indicating that abiotic iodine speciation processes occur inside MBL particles, independently from algal photochemistry.

3. Radioactive iodine in the marine environment

From the viewpoint of global iodine circulation, the introduction of radioisotopes by human activities is likely to have an impact on the environment [58]. Iodine has 33 radioactive isotopes. The main iodine radioisotopes generated by human activities related to nuclear energy are ^{131}I (half-life of 8.04 days) and ^{129}I (half-life of 15.7 × 10^6 years). ^{131}I is a fission by-product of uranium 235 generated in power plants to produce electricity. Because of its rapid decay, it is also used for medical purposes (diagnosis and radiotherapy). ^{129}I is also produced in power plants, its very long half-life making it the major remaining iodine radioisotope of the nuclear fuel cycle [59,60]. Other sources of radioactive iodine include medical uses of ^{131}I and natural production of ^{129}I, fallout from atmospheric nuclear weapon testing and major nuclear power plant accidents (Chernobyl in spring 1986).

In 1999, ^{129}I controlled liquid wastes discharges by the nuclear fuel reprocessing plant of COGEMA[1] La Hague in the English Channel reached 1.8 TBq and remained above 1 TBq per year until 2004. Beside control samplings by COGEMA, two brown seaweed species were periodically sampled by IRSN[2] between 2002 and 2005 on the French shores of the English Channel, and analyzed for ^{129}I mass activities. In the vicinity (~ 5 km) of the pipe outlet of the COGEMA La Hague reprocessing plant (Goury), ranges were 20–150 Bq kg^{-1} dry weight (dw) in *Fucus serratus* and 80–740 Bq kg^{-1} dw in *L. digitata*. From the point of release, liquid wastes discharges dispersion towards the Eastern English Channel, according to hydrodynamics is well documented (see [61] and references herein for details). In Brittany (Roscoff, Western English Channel), ^{129}I was not detected (< 2 Bq kg^{-1} dw) in *F. serratus* whilst in the Straits of Dover (Wimereux), values ranged from 5 to 21 Bq kg^{-1} dw. Laminariales are also exploited to extract alginates, and are used in food industry and cosmetics, or directly in agriculture as fertilizing agents. Transfer of radionuclides released in the marine environment towards alginate extraction products was investigated and the conclusions were that more than 95% of ^{129}I present in the initial product (seaweed) were eliminated in the end products [62,63]. In addition to their key roles in iodine cycling in the marine environment, macroalgae are an important feeding source for a number of marine herbivores [64], thus representing a major initial trophic level in the food web. A clear understanding of the metabolism of iodine in

[1] COGEMA : COmpagnie GEnérale des MAtières nucléaires (AREVA).
[2] IRSN: Institute for Radioprotection and Nuclear Safety.

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

brown algae as well as their possible labeling by radioactive iodine from nuclear industry is thus needed.

4. Physiology of iodine transfers in a model species *L. digitata*

4.1. Iodine speciation and uptake in L. digitata

Iodine is known to accumulate in brown seaweeds in a concentration range which does not compare with other biological systems [14,65]. Individual iodine levels in *L. digitata* sporophytes from natural populations range from 0.3% dw in the largest plants to a maximum of 4.5% dw in juvenile plantlets [66]. These contents represent a 30,000–150,000-fold accumulation of this element from seawater. In this perennial species, iodine contents increase from the younger meristematic zone to the older distal parts of the blade and from the stipe towards the holdfast [67]. In the annual *L. saccharina*, however, iodine appears to be translocated towards the meristematic zone [68].

In the Laminariales, ancient reports indicate that iodine is mostly stored as inorganic iodine which amounts to 80–90% of the total iodine content, the remainder consisting of the iodinated amino-acids mono- and di-iodotyrosine [69–72]. Recent investigations using anion-exchange chromatography coupled to ICP-mass spectrometry (MS) confirmed that the most predominant inorganic iodine species present in commercial brown seaweeds is iodide and also revealed the presence of monoiodotyrosine and di-iodotyrosine in protein fractions of the brown alga *Undaria pinnatifida* [73]. Moreover, X-ray absorption spectroscopy (EXAFS) studies have detected a low iodine incorporation into Tyr residues of haloperoxidase from *Ascophyllum nodosum* (see part 5) [74]. However, the presence of iodide as the major form inside the cells implies that a huge transmembrane electrochemical gradient exists (the membrane is supposed to be polarized and negatively charged inside). Therefore, understanding the transport mechanism responsible for the net iodine uptake in *Laminaria* cells represents a real challenge for physiologists. Whether iodide is homogeneously distributed in the cytosol or trapped in internal cell structures just displaces the question from the plasma membrane to another intracellular membrane. According to early cytological studies using cresyl blue stain, iodine is believed to be localized in non-specialized vacuoles in the blade cortical cells and in phenolics-containing physodes in stipe cells [49,68,75].

In seawater, iodide spontaneously reacts with hydrogen peroxide to produce hypoiodous acid (HIO) and this oxidation reaction is slow (Fig. 2) [76]. It was originally observed that oxidation of iodide obviously occurs very efficiently at *Laminaria* cell surface [47–49]. Kylin (1929) proposed that an "iodine oxidase" was responsible for the catalyzed oxidation of iodine and that I_2 was finally taken up in the algae [49]. The key role of iodine oxidation in its uptake was pointed out later by Shaw [71,72]. Indeed, both HIO and I_2 are more lipophilic than I^- anion and may diffuse across the membranes lipid bilayer. More recently, Küpper et al. (1998) [14] confirmed that apoplastic oxidation of iodide increased iodine permeability in *Laminaria*, and underlined the key role of vanadium-dependent haloperoxidases in these processes. In *L. digitata* the occurrence of such an enzyme, specialized in the oxidation of iodide (see part 5), should explain its high efficiency for iodine accumulation, as compared to other algae [77,78]. The mechanism of iodine uptake in *L. digitata* formerly suggested by Shaw [72] and refined by Küpper et al. [14] is depicted in Fig. 2.

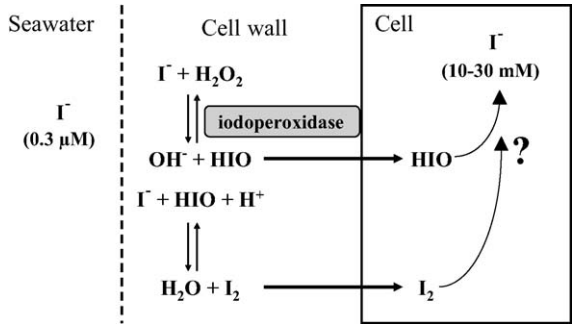

Fig. 2. Mechanisms of iodine uptake in *L. digitata* cells, involving vanadium-dependent iodoperoxidase (adapted from Refs. [14,72]). The reduction processes of HIO or I_2 in the cytosol are still unknown.

This model deserves a few comments: The cell plasma membrane must be impermeable enough to iodide to prevent overleaking along the huge electrochemical gradient. The iodine uptake mechanism must be efficient enough to counterbalance iodide leaking and a little bit more to account for iodovolatilization as well as growth of the seaweed. Transformation of seawater iodide into oxidized iodine species (HIO, I_2) increases its permeability and those oxidized forms may diffuse across the plasma membrane, provided their chemical gradient is favorable (as explained below in terms of chemistry and thermodynamics). Therefore, maintaining this favorable inward gradient is obviously a key issue to achieve iodine net uptake. The concentrations of oxidized iodine forms outside the cell are presumably low since they are in a chemical equilibrium with iodide and to maintain the favorable inward gradient, the concentrations inside must be kept lower. This means that reducing iodine back to iodide into the cells must be very efficient. In other words, each time an iodide leaks out of the cells or is lost through iodovolatilization, an I_2 or HIO molecule must enter the cell and be reduced to replace that departed iodide ion and hence ensure homeostasis. Thus the question arises as to whether the intracellular reducing capacity is sufficient to achieve this mechanism.

4.2. Iodine release by L. digitata

In response to various biotic and abiotic stresses, marine algae are known to produce volatile halocarbons [19,34,50,79]. When incubated in the presence of alginate guluronate blocks *L. digitata* responds by a strong oxidative burst [17], a challenge which likely mimics an alteration of the cell wall by micro-organisms. In the presence of exogenously-added H_2O_2, an increase in radioactive iodine efflux was observed [14], and a net iodine efflux was also induced by oligoguluronate elicita-

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

tion (Fievet et al., unpublished results). It was proposed that both the oxidative burst and the concomitant iodine efflux take part in the early defense responses by the algae. More recently, these two oxidative stresses, as well as ozone or UV treatments were shown to induce a release of volatile iodocarbons and of molecular iodine in *L. digitata* [19,34]. The liberation of iodide ions outside the cells could detoxify hydrogen peroxide and the presence of haloperoxidases in the apoplasm could further catalyze the production of extracellular iodinated organic compounds, capable of repelling micro-organisms [37]. This appealing hypothesis (schematized in Fig. 3) raises the question of the transport mechanisms which would mediate this iodine efflux. Indeed the problem is no longer the accumulation of iodine against a considerable gradient but on the contrary the subtle control of membrane permeability to let iodide transiently leak out from the cell without depriving the preciously built cellular iodine pool.

Two complementary techniques may contribute to explore the transport mechanisms of iodine in *L. digitata*. Unidirectional fluxes measurements using iodine radioisotopes as tracers were already used in many experiments [14,71]. They provided valuable information on the changes in membrane permeability to iodine, and in particular on the effects of iodide oxidation mediated by haloperoxidases. Indeed, turning poorly permeable iodide into more permeable oxidized iodine results in an increase of the overall iodine permeability, which is reflected by an increased radioactive iodine uptake. But this technique does not discriminate between an increase in net iodine movements and an increase in the exchange rate between radioactive iodine and stable iodine. Net iodine movements could be calculated from the determinations of both unidirectional iodine influx and outflux, as traced by radioactive iodine under the same conditions. But this would require a very precise knowledge of intracellular iodine concentration, which is not easy to monitor. Another approach consists in measuring net iodine movements directly by following iodine concentration in seawater, starting with a suitable ratio between the external medium volume and the seaweed mass. Thus a decrease in iodine concentration in seawater means a net uptake and/or iodovolatilization by the algae. Conversely an increase in seawater means a leakage of iodine by the algae, whereas no change outside just means that iodine loss is just counterbalanced by uptake. The combination of these two techniques allows investigating simultaneously both iodine permeability and iodine net transport across the cell membrane, and should be completed as far as possible with measurements of volatile halogenated compounds in both in seawater and in the gaseous phase. Two experimental conditions may be considered: (i) control condition where the seaweed has to maintain homoeostasis (uptake counterbalancing leak, iodovolatilization and growth) and (ii) response to oxidative stress which is expected to induce iodine movements and increase in the production of volatile iodine organic compounds [19].

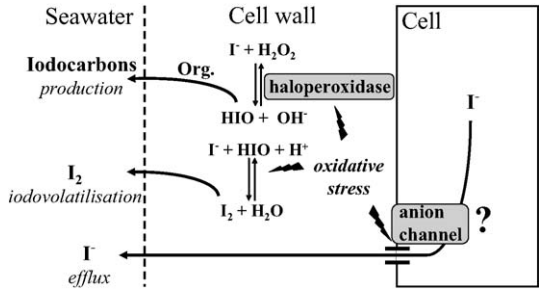

Fig. 3. Schematic representation of iodine releases in *L. digitata* cells, in response to oxidative stress, showing iodide efflux, iodovolatilization and iodocarbon production with the involvement of vanadium-dependent haloperoxidases. Org.: organic compound.

5. Vanadium-dependent haloperoxidases in marine macroalgae

As pointed out below, vanadium-dependent haloperoxidases are likely to play a central role both in the iodine uptake and in the production of volatile halocarbons in algae [14–16]. Haloperoxidases catalyze the oxidation of halides in presence of hydrogen peroxide and they are named according to the most electronegative halide that they can oxidize, i.e. chloroperoxidases (CPOs) can catalyze the oxidation of chloride as well as of bromide and iodide, bromoperoxidases (BPOs) react with bromide and iodide, whereas iodoperoxidases (IPOs) are specific of iodide.

5.1. Occurrence and biochemical characterization

The first vanadium-dependent haloperoxidase was discovered in *A. nodosum*, a brown alga belonging to the Fucales [80]. Since, vHPO activities have been detected in a very large number of red and brown macroalgae and most of them have been identified as bromoperoxidases (for review [81]). If *o*-dianisidine detection assay has been used for qualitative characterization of vHPOs on native electrophoretic gels [82], bromoperoxidase specific activities have been measured using standard monochlorodimedone (MCD) assay. The values reported are extremely variable, i.e. from 12 units mg^{-1} of proteins in *L. saccharina* [83] to 1730 units mg^{-1} in *Macrocystis pyrifera* [84]. However, given the sensitivity of MCD assay, especially with pH variations (see part 6), qualitative comparisons between different sources of vBPOs seem to be difficult. A weak chloroperoxidase activity has been reported using the same assay for the so-called vBPO from *A. nodosum*, but with very high concentrations of chloride [85]. Few species belonging to Laminariales [77,83] feature strict iodoperoxidase activities, i.e. enzymes showing no reaction to the MCD assay and in which in vitro formation of triiodide has been measured (see part 6).

Up to now, few data are available on vHPO biochemical properties, owing to the tedious purification procedures of these enzymes from algal matrices that are extremely rich in anionic polysaccharides and polyphenolic compounds. Concerning the Phaeophyceae, the improvement of aqueous two-phase extraction protocols for Laminariales and Fucales [86] allowed the acquisition of biochemical data on some partially or fully purified enzymes. Whereas the molecular masses of

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

Table 1
Molecular characterized vanadium-dependent haloperoxidases in marine algae

Species	Activity	GenBank Accession	X-ray structure	Mutants	References
A. nodosum	vBPO	P81701[a]	1QI9[b]	–	[91]
F. distichus	vBPO	AAC35279	–	–	[92]
L. digitata	vBPO	CAD37191–2	–	–	[77]
	vIPO	CAF04025	–	–	[78]
C. officinalis	vBPO	1QHBA-F[a]	1QHB[b]	–	[87]
		AAM46061	–	H480A[d]	[93]
C. pilulifera	vBPO	BAA31261–2	1UP8[c]	R397X[d]	[88,94–96]

vBPO: vanadium-dependent bromoperoxidase; vIPO: vanadium-dependent iodoperoxidase; X: all amino-acids except R.
[a] Obtained by protein sequencing.
[b] Resolution of the crystal structure from the native protein.
[c] From the recombinant protein.
[d] Residue numbering according to the translated cDNAs.

denaturated proteins are quite similar, i.e. around 65 kDa, the native enzymes consist of dimeric assemblies, ranging from 100 to 800 kDa. For instance, in the red alga *Corallina pilulifera*, vBPOs are organized in dodecamers [87,88]. In *L. digitata*, vBPOs consist of dimers which in solution spontaneously self-associate, and the aggregation into forms with a higher molecular mass is likely to be a common tendency for brown algal haloperoxidases [77,83].

In contrast to heme-haloperoxidases, vHPO activities are relatively resistant to high temperature exposures and the enzymes are still active in oxidative conditions and in the presence of different organic solvents such as acetone, methanol or ethanol (for reviews, [81,89]). These properties as well as the ability to halogenate a broad range of organic compounds of both commercial and pharmaceutical interests make vHPOs good candidates for use in industrial biotransformations [90].

5.2. Molecular and structural characterization

At the molecular level, four closely-related cDNAs are available for *Corallina* sp. vBPOs, whereas four cDNAs of vBPOs have been cloned in the three following brown algae, *A. nodosum*, *Fucus distichus* and *L. digitata* (Table 1). In the latter species, a full-length cDNA has been shown to encode a vIPO enzyme. Up to now, X-ray crystal structures have been solved for the vBPOs from the red algae and from *A. nodosum*.

The global dimeric structure of algal vBPOs is extremely conserved, folding into α-helices with a few short β-strands. The main tertiary structural motif of two four-helix bundles is similar to the reported X-ray crystal structure of the vCPO from a terrestrial fungi, *Curvularia inaequalis* ([97], for review [16]). The surfaces of the two monomers are tightly bound one to another by hydrophobic interactions and in the case of *A. nodosum* vBPO two additional inter-molecular disulfide bridges are also involved in this interface. Very recently, EXAFS and MS studies have led to partially reassess the electron density map of the brown algal vBPO structure, showing post-translational bromination and iodination of tyrosine residues at the surface of *A. nodosum* vBPO [74].

The vanadium-binding site motif, HP[S/A]Y[P/G][S/A]GHA, is relatively well conserved in all algal vHPO protein sequences [78]. Despite some differences, the protein scaffold around the vanadium cofactor (*A. nodosum*) or the inorganic phosphate substitute (*Corallina officinalis* and *C. pilulifera*) is very similar between the crystallized vBPOs and the vCPO of *C. inaequalis* (Fig. 4).

The vanadium cofactor is coordinated by four non-protein oxygen atoms and one nitrogen (Nε2) atom from a histidine residue into trigonal bipyrimidal geometry. Based on X-ray

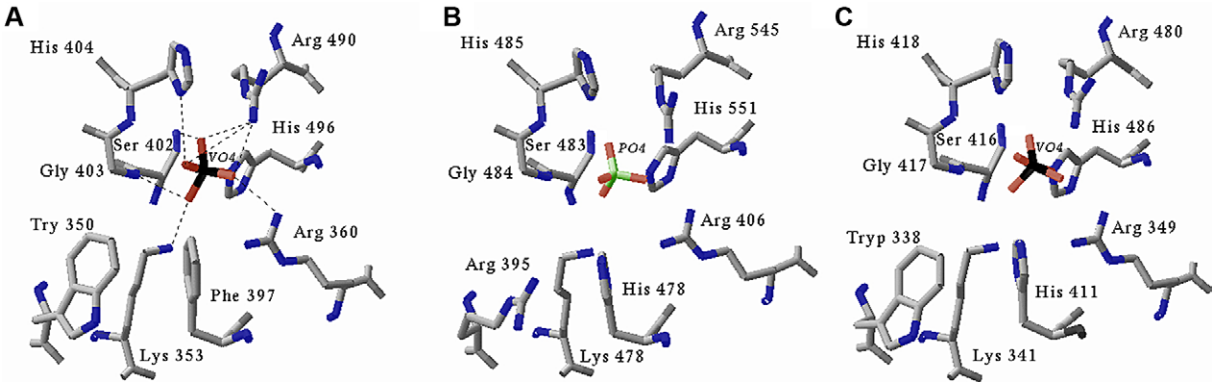

Fig. 4. The vanadate-binding site structure of vHPOs. (A) *C. inaequalis* vCPO (PDB accession no. 1VNC), (B) *C. officinalis* vBPO (PDB accession no. 1QHB, note that the numbering is based on protein sequence of *C. officinalis*), (C) *A. nodosum* vBPO (PDB accession no. 1QI9). Residues are drawn in atom colors. Vanadate (A and C) and the inorganic phosphate (B) are represented in the center of the picture and the hydrogen-bonding network, conserved in all three vHPOs, is indicated by dashed lines around the vanadate center (A).

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

data, the linkage between the metal and the histidine ligand was first assigned as a direct coordinating bond [97,98] and later as a covalent bond [91,99,100]. The hydrogen-vanadate ($HOVO_3^{2-}$) is stabilized into a hydrogen-bonding network, involving six highly conserved residues (one apical histidine, two arginine, a lysine, a glycine and a serine). Kinetics and structural studies of single site mutants of vCPO from *C. curvularia* have shown the importance of these residues in binding vanadate, but also the rigidity of the active site, resulting from a large number of hydrogen bonding interactions around [100]. It is interesting to note that the only vHPO presenting a substitution among these six residues (the serine is replaced by an alanine) is the vIPO from *L. digitata*, and its fine structure is still unknown [78].

5.3. The catalytic cycle

Detailed steady-state kinetic analyses have suggested a bi–bi ping–pong two-substrate mechanism, in which hydrogen peroxide first binds the vanadate active site, followed by halide oxidation (for reviews [16,89]). For these studies, the *C. inaequalis* vCPO and the *A. nodosum* vBPO have served as main models to understand catalytic mechanisms. However, high conservation of the residues at the catalytic center and phylogenetic analyses strongly suggest that all vHPOs share the same mechanism of action [78].

The first step of the catalytic cycle consists in the coordination of hydrogen peroxide by bound hydrogen-vanadate, producing a square-based pyramidal peroxovanadium complex, from which the X-ray structure of vCPO has been resolved [98]. X-ray absorption and RMN spectroscopy studies have also detected the H_2O_2 effect on the vanadium active site structure and the presence of a peroxovanadium intermediate for *A. nodosum* vBPO [101–104]. In addition, this model is consistent with further mutagenesis and steady state kinetic analyses on fungal vCPO [99,105,106] and on red algal vBPOs [93,95,96].

Although the interaction between H_2O_2 and the bound hydrogen-vanadate is widely accepted for vHPOs, the subsequent steps of the catalytic cycle are not precisely elucidated. The halide substrate is not directly coordinated by the peroxovanadium intermediate, as established by X-ray absorption spectroscopy for *A. nodosum* vBPO [101,102,104]. Therefore, a halide fixation site in the vicinity of the active site has been suggested but never demonstrated. The peroxovanadium complex oxidizes X^- to produce a diffusible halogen intermediate (X_2 and XOH) which has not been clearly identified. The nature of this first oxidized entity is still doubtful since X_2 and XOH are in spontaneous equilibrium (Eq. (1), Fig. 6) [107] and both can account for the halogenation/oxygen insertion properties of the vHPO/H_2O_2/X^- system. Although XOH is probably initially produced through a two-electron oxidation [108], the reaction product is generally considered as a mixture of different species ("X^+" = XOH, X_2, X_3^-). In the absence of an organic acceptor, a second equivalent of H_2O_2 is oxidized

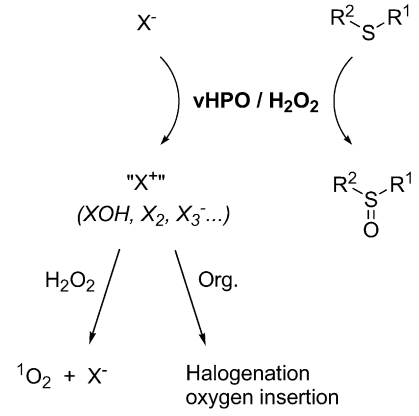

Fig. 5. Reactions of the vHPO/H_2O_2 system. X: Br, Cl, I. Org.: organic compound. R^1, R^2: alkyl, aryl.

$$I_2 + H_2O \rightleftharpoons IOH + I^- + H^+ \quad (1)$$

$$I_2 + I^- \rightleftharpoons I_3^- \quad (2)$$

$$3IOH \rightleftharpoons IO_3^- + 2I^- + 3H^+ \quad (3)$$

$$I^- + H_2O_2 + H^+ \rightleftharpoons IOH + H_2O \quad (4)$$

$$IOH + H_2O_2 \rightleftharpoons I^- + H^+ + O_2 + H_2O \quad (5)$$

Fig. 6. Main reactions of iodine species in solution.

by "X^+", resulting in the formation of singlet dioxygen and X^- (Fig. 5) [109].

Thanks to the "X^+" species, vHPOs can catalyze the halogenation and/or oxygen insertion of various organic substrates such as phenols, ketones, indoles, terpenes and sulfides (see Fig. 5) [16]. Moreover, the vBPO/H_2O_2/Br^- system catalyses regioselective brominations of indole derivatives, suggesting the fixation of an organic substrate near the active site and/or the production of a non-freely diffusible halogenating intermediate (an enzyme-bound halogen species) [110,111]. With regard to the vHPO/H_2O_2/I^- system, no study of iodination/oxygen insertion of organic substrates has been carried out, though the oxidized species of iodine are reactive towards phenols and electron rich heterocycles.

In the absence of halides, vBPOs catalyze the enantioselective oxidation of sulfides into sulfoxides (Fig. 5) [112]. These sulfoxidation reactions occur at the vanadium binding site through a direct oxygen transfer from the peroxovanadium intermediate to the sulfide [113].

6. Iodide chemistry in solution: implication for vIPO biochemistry

The determination of enzymatic activity is central for the understanding of structure, functions and mechanism of action of an enzyme. Most assays are based on the determination of substrate consumption and/or product formation rates using spectrophotometric methods. Haloperoxidases differ from other enzymes by the production of a very reactive intermediate which is undetectable using standard methodologies. This

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

intermediate undergoes spontaneous redistribution to produce a mixture of halogen species. Standard assays for the determination of HPO activity are based on the reactivity of this mixture. The speciation of such a mixture is strongly dependent on pH and total iodine species concentration. A slight variation in the assay conditions leads to significant changes in the nature and distribution of the iodinating mixture, and may lead to result misinterpretation. Thus, it is necessary to define the reactivity of iodine species that are involved in standard conditions for vIPO activity determination.

6.1. Iodine chemistry in solution

Iodine chemistry is unique due to complex and interconnected reactions that produce numerous iodine entities. Iodine species distribution in aqueous solutions is strongly influenced by pH, total "I" concentration, and redox state of the solution. This complexity is illustrated by the spectacular behavior of the oscillating iodine–clock reaction (Briggs–Rauscher reaction) [114,115]. In mild conditions ($4 < pH < 10$), five reactions dominate in solution (Fig. 6, Eqs. (1–5)).

Gottardi reported the distribution of iodine species in aqueous solutions at different pH, I_2 and I^- concentrations [116]. The author showed that IO_3^- formation rate (Eq. (3), Fig. 6) is negligible at acidic pH and becomes significant at $pH > 7$. In addition, the chemical oxidation of H_2O_2 to O_2 by IOH (Eq. (5)) or I_2 (Eq. (1) + Eq. (5), Fig. 6) has been known for a long time [117]. Reaction rates of Eq. (5) increase with pH up to significant dioxygen production in alkaline solutions. On the other hand, hydrogen peroxide is capable of oxidizing I^- to IOH (Eq. (4)) at rates increasing with H^+ concentration. Thus the mixture of I^- and H_2O_2 represents a pseudo auto-catalyzed redox system whose equilibrium depends strongly on pH conditions. The degradation of H_2O_2 is minimal at pH within 5–7.

The triiodide formation is the result of I_2 and I^- complexation according to Eq. (2) [107]. As the equilibrium constant of triiodide formation is small ($K_{eq2} = 830\ M^{-1}$), I_2/I^- complexation is weak in diluted solutions. Free I_2, I^-, and I_3^- concentrations can be calculated from K_{eq2} for various $[I^-]_{tot}$, and $[I_2]_{tot}$ At fixed $[I^-]_{tot}/[I_2]_{tot}$, the percentage of complexed I_2 strongly varies and is quasi-total only for $[I^-] > 10^{-2}$ M (Fig. 7). This example illustrates the lack of correlation between $[I_2]$ and $[I_3^-]$ throughout a practical range of $[I^-]$.

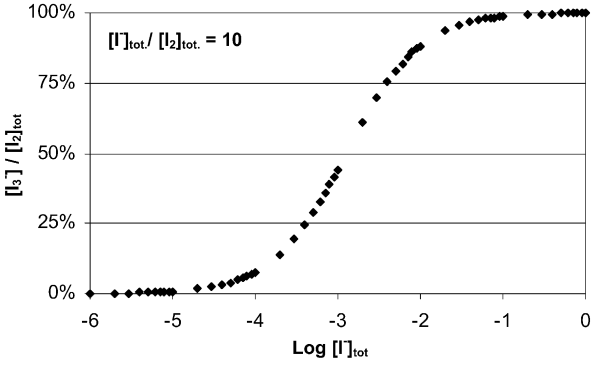

Fig. 7. Percentage of I_2/I^- complexation at various $[I^-]_{tot}$, $[I^-]_{tot}/[I_2]_{tot} = 10$. Concentrations are expressed in M.

It is also important to note that triiodide equilibrium is dependent on solvent nature. A strong stabilization of I_3^- occurs when solvent is added to the solution ($K_{eq2} = 830\ M^{-1}$ in H_2O and 25,000 M^{-1} in 2-propanol/water = 7/3) [118]. As a result, I_3^- is formed at much lower iodide concentration in alcoholic mixtures. In addition, the molar absorption coefficient of I_3^- at 350 nm drops from 25,000 $M^{-1}\ cm^{-1}$ in pure water to 14,000 $M^{-1}\ cm^{-1}$ in a 2-propanol/water mixture (v/v = 7/3), and is accompanied by a shift in λ_{max} (350–357 nm). This spectrophotometric property is explained by I_3^- symmetry breaking induced by solvent polarity [119,120].

6.2. Limitations of vHPO activity assays

A standard assay for measuring bromo- and chloroperoxidase activities is based on the halogenation of monochlorodimedone (MCD) [121]. MCD is a 1,3-diketone with an activated carbon that can be brominated or chlorinated by XOH or X_2. The reaction is followed spectrophotometrically at 290 nm by monitoring the loss of absorbance of MCD ($\varepsilon = 20{,}000\ M^{-1}\ cm^{-1}$). The MCD formation was shown to be quantitative (with respect to H_2O_2 consumption) only at $pH < 6.5$ [108]. This can be explained by enhanced dioxygen formation (Eq. (5), Fig. 6) in alcaline solutions, as well as reduced MCD halogenation rates at near-neutral pH, in agreement with the general reactivity of ketones toward electrophiles. BPO and CPO quantitative activity measurements are thus limited to acidic pH. An alternative assay consists in the halogenation of phenol red [122]. This test has only been used for qualitative purpose due to polyhalogenation of the dye and because it is restricted to a very sharp range of pH ($6 < pH < 7$).

The MCD assay is not adapted to measure iodoperoxidase activity due to the poor reactivity of "I^+" toward MCD and/or a high instability of the iodinated product (which can revert back into MCD and iodide). The determination of vIPO activity is only based on the triiodide spectrophotometric detection. Triiodide is produced from reactions 1 and 2 (Fig. 6). Reactions 3–5 are side-reactions that will lead to non-stoechiometric triodide formation. Whichever IOH or I_2 is first produced by the vIPO/H_2O_2/I^- system, spontaneous redistribution occurs according to Eq. (1) (Fig. 6) up to virtually total I_2 formation ($K_{eq1} = 6.8 \times 10^{-13}\ M^2$ according to Eigen and Kustin [107]). Then molecular iodine (I_2) complexes with I^- to form triiodide (Eq. (2), Fig. 6). At pHs and initial iodide concentration generally used for vIPO activity measurement (i.e. $pH < 7$, $[I^-]_{tot} = 2$–10 mM, [81]), the "I^+" mixture is considered to be dominated by I_3^-. Triiodide is then quantified spectrophotometrically ($\varepsilon = 25{,}000\ M^{-1}\ cm^{-1}$ at 350 nm). Unfortunately, this method is not free of intrinsic problems. For quantitative IPO activity assay, the stoechiometry of H_2O_2 consumption versus I_3^- production must be ensured. We and others have observed that for the IPO/H_2O_2/I^- system, this condition is never reached [123]. Further experiments showed that non-enzymatic I_3^- pro-

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

duction was very sensitive to H^+ concentration and is optimal at pH within 5 to 6.5. This non-stoechiometric I^- oxidation can be explained by significant non-enzymatic H_2O_2 degradation according to Eqs. (4) and (5) (Fig. 6), as well as potential IO_3^- production (Eq. (3), Fig. 6). Moreover, it is usually observed that the IPO-mediated triiodide formation is negligible below the millimolar range of I^-. This result should not be interpreted as a lack of IPO activity but rather as a consequence of poor I_2/I^- association. Thus IPO activity measurement is restricted to high iodide concentrations, in disagreement with in vivo conditions.

In conclusion, whereas these two assays are the only ones available to date for steady-state studies of vHPO, they present intrinsic problems. Due to high pH sensitivity, qualitative vHPO activity determinations with both the MCD and triiodide assays are pertinent only in acidic solutions. The determination of an optimum pH for vHPO is then questionable using these assays. Moreover, the IPO activity determination by the triiodide assay is not strictly quantitative avoiding K_M and V_{max} determination and enzymology analyses.

6.3. New insights on vHPO selectivity

Once different types of enzymes have been characterized, i.e. first vCPO and vBPO, and more recently vIPO, structural and biochemical researches have been focused on residues to the vicinity of the active site, which could explain the origin of halide selectivity. As the halide is not directly bound to the vanadate, the surrounding amino-acids, which were different between vHPOs, were suspected to play this role. However, mutagenesis experiments have not been able to clearly identify one residue responsible for halide specificity [89,93,96,106]. For instance, in *C. inaequalis* vCPO, the serine residue which is hydrogen bound to the vanadate cofactor has been mutated to alanine [106], leading to a putative conformation similar to *L. digitata* vIPO [78]. However, this mutation significantly decreased chloride oxidation, but had minor effects on bromination kinetic parameters [106]. A consensus hypothesis is starting to emerge, i.e. instead of a unique specific binding site for halide, halogen specificity would involve many factors such as the hydrogen bonding network in the active site, as well as the redox state and the distribution of charges of vanadate center [78,93,106].

Indeed, a significant difference between halogens lies on the reduction potentials of XOH and X_2 species, which decrease in the same order, i.e. Cl > Br > I (Table 2). If the peroxovanadium center of vIPO has a low enough redox potential, bromide and chloride oxidation will be chemically unfavorable. From a molecular point of view, little differences in amino-acid residues and in stabilization of peroxovanadium intermediate may lead to lowered redox potential, a condition that is sufficient to account for halide discrimination.

Table 2
Redox potentials of XOH/X^- and X_2/X^- at pH 7.8 [124]

Halogen	Redox potential ($E_{1/2}$) at pH 7.8	
	$X_2 + 2e^- = 2X^-$	$XOH + H^+ + 2e^- = X^- + H_2O$
Cl	+1.36 V	+1.25 V*
Br	+1.07 V	+1.10 V*
I	+0.54 V	+0.76 V*

* Calculated from standard redox potential using the Nernst equation.

7. Concluding remarks and future research fields

Preliminary measurements on volatile iodinated compounds generated by *L. digitata*, either under controlled steady state conditions or in response to oxidative stresses, suggested they represent a small part of the overall iodine transport budget, compared to iodide movements (involving oxidation/reduction cycling). However, this needs to be confirmed with special attention to I_2, which has not been precisely quantified so far from stressed algae. Recent studies have indeed underlined the important involvement of I_2 in marine aerosol formation in relationships to climatic consequences worldwide [10,11,53,125]. Investigations using non-destructive EXAFS (Küpper et al., unpublished results), trace element imaging by X-ray microprobe [126] and high-resolution analytical techniques such as ICP-MS coupled with capillary GC by Schwarz and Heumann [127], will definitely help to refine our knowledge of iodine speciation and distribution in brown algae, and especially during stress responses.

As shown in response to oligoalginate elicitation in *L. digitata*, a transient increase in iodide anions permeability could be mediated by e.g. the opening of anion channels. Because of the presence of coating alginates, access to the plasma membrane and use of electrophysiology techniques are difficult in *L. digitata* cells. However, the pharmacology of anion channels should be tested on flux experiments. The characterization of an iodine-specific haloperoxidase provides new keys to understand its role in the extreme efficiency of iodine accumulation in kelps, by reconstituting in vitro and in vivo uptake systems. The reverse mechanism of iodine intracellular reduction found in *L. digitata* definitely deserves specific attention as a central clue to its whole halogen metabolism.

In particular oxidative conditions, a number of physiological pathways are likely to involve the intracellular iodine pool of kelps. In *L. digitata*, vHPO are encoded by distinct multigenic families, which members should have evolved towards different specialized functions, such as iodine uptake [77,78], chemical defense and anti-fouling processes through halocarbon production [79,128,129], oxidative protection [130], and cell wall assembly [130,131]. Molecular and structural characterization of these enzymes will help studying their biochemical specificity, their regulation and their subcellular localization, in order to understand the special role of haloperoxidases in the trafficking of iodine through the cell.

Concerning kinetic aspects, it is crucial to design new chemical probes for monitoring both "I^+" and "Br^+" species during the enzymatic reaction. Moreover, for physiological studies of iodine transfers, substantial progress definitely lies in the identification of new chemicals specifically interacting with vHPOs. Finally, these specific substrates and inhibitors will also provide useful tools to further analyze high-resolution

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

structure of vIPO complexes and to study structure/function relationships of these enzymes. Altogether, these combined approaches would lead to a better understanding of the catalytic mechanism of iodination and of iodine transport in kelps.

Acknowledgements

This work was supported in part by CNRS, IRSN, CEA; E.V. and E.T. were supported by Ph.D. and post-doctoral fellowships, respectively, from the TOXNUC-E program.

References

[1] C.C. Johnson, The Geochemistry of Iodine and Its Application to Environmental Strategies for Reducing the Risks from Iodine Deficiency Disorders, British Geological Survey, Commissioned Report, CR/03/057N, Keyworth, Nottingham, 2003 (54 pp).

[2] C.C. Johnson, F.M. Fordyce, A.G. Stewart, Environmental controls in Iodine Deficiency Disorders, Project Summary Report, British Geological Survey, Commissioned Report CR/03/058N, Keyworth, Nottingham, 2003 (20 pp).

[3] D.C. Whitehead, The distribution and transformations of iodine in the environment, Environ. Int. 10 (1984) 321–339.

[4] B. Giese, F. Laturnus, F.C. Adams, C. Wiencke, Release of volatile C1–C4 by marine macroalgae from various climate zones, Environ. Sci. Technol. 33 (1999) 2432–2439.

[5] R.M. Moore, W. Groszko, Methyl iodide distribution in the ocean and fluxes to the atmosphere, J. Geophys. Res. 104 (1999) 11163–11171.

[6] K.R. Redeker, N. Wang, J.C. Low, A. McMillan, S.C. Tyler, R.J. Cicerone, Emissions of methyl halides and methane from rice paddies, Science 290 (2000) 966–969.

[7] S. Amachi, M. Kasahara, S. Hanada, Y. Kamagata, H. Shinoyama, T. Fujii, Y. Muramatsu, Microbial participation in iodine volatilization from soils, Environ. Sci. Technol. 37 (2003) 3885–3890.

[8] C.E. Jones, L.J. Carpenter, Solar photolysis of CH_2I_2, CH_2ICl, and CH_2IBr in water, saltwater, and seawater, Environ. Sci. Technol. 39 (2005) 6130–6137.

[9] L.J. Carpenter, J.R. Hopkins, C.E. Jones, A.C. Lewis, R. Parthipan, D.J. Wevill, L. Poissant, M. Pilote, P. Constant, Abiotic source of reactive organic halogens in the sub-arctic atmosphere?, Environ. Sci. Technol. 39 (2005) 8812–8816.

[10] L.J. Carpenter, Iodine in the marine boundary layer, Chem. Rev. 103 (2003) 4953–4962.

[11] C.D. O'Dowd, T. Hoffmann, Coastal new particle formation: a review of the current state-of-the-art, Environ. Chem. 2 (2005) 245–255.

[12] R. von Glasow, Seaweed, iodine, new particles and atmospheric chemistry—the current state of play, Environ. Chem. 2 (2005) 243–244.

[13] M.B. Courtois, Sur un nouvel acide formé avec la substance découverte par M. Courtois, Ann. Chim. Phys. (Paris) 88 (1813) 311–318.

[14] F.C. Küpper, N. Schweigert, E. Ar Gall, J.-M. Legendre, H. Vilter, B. Kloareg, Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide, Planta 207 (1998) 163–171.

[15] R. Wever, M.G.M. Tromp, B.E. Krenn, A. Marjani, M. Van Tol, Brominating activity of the seaweed *Ascophyllum nodosum*: impact on the biosphere, Environ. Sci. Technol. 25 (1991) 446–449.

[16] A. Butler, J.N. Carter-Franklin, The role of vanadium bromoperoxidase in the biosynthesis of halogenated marine natural products, Nat. Prod. Rep. 21 (2004) 180–188.

[17] F.C. Küpper, B. Kloareg, J. Guern, P. Potin, Oligoalginates elicit an oxidative burst in the brown alga Kelp *Laminaria digitata*, Plant Physiol. 125 (2001) 278–291.

[18] F.C. Küpper, D.G. Müller, A.F. Peters, B. Kloareg, P. Potin, Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of sporophytes of Laminariales, J. Chem. Ecol. 28 (2002) 2057–2081.

[19] C.J. Palmer, T.L. Anders, L.J. Carpenter, F.C. Küpper, G. McFiggans, Iodine and halocarbon response of *Laminaria digitata* to oxidative stress and links to atmospheric new particle production, Environ. Chem. 2 (2005) 282–290.

[20] G.T.F. Wong, The marine geochemistry of iodine, Rev. Aquat. Sci. 4 (1991) 45–73.

[21] J.S. Edmonds, M. Morita, The determination of iodine species in environmental and biological samples, Pure Appl. Chem. 70 (1998) 1567–1584.

[22] R.M. Moore, O.C. Zafiriou, Photochemical production of methyl iodide in seawater, J. Geophys. Res. 99 (1994) 16415–16420.

[23] J.D. Happell, D.W.R. Wallace, Methyl iodide in the Greenland/Norwegian seas and the tropical Atlantic ocean: evidence for photochemical production, Geophys. Res. Lett. 23 (1996) 2105–2108.

[24] F. Keppler, R. Eiden, V. Niedan, J. Pracht, H.F. Schöler, Halocarbons produced by natural oxidation processes during degradation of organic matter, Nature 403 (2000) 298–301.

[25] P.M. Gschwend, J.K. MacFarlane, K.A. Newman, Volatile halogenated organic compounds released to seawater from temperate marine macroalgae, Science 227 (1985) 1033–1035.

[26] S.L. Manley, M.N. Dastoor, Methyl halide (CH_3X) production from the giant kelp *Macrocystis*, and estimates of global CH_3X production by Kelp, Limnol. Oceanogr. 32 (1987) 709–715.

[27] S.L. Manley, M.N. Dastoor, Methyl iodide production by kelp and associated microbes, Mar. Biol. 98 (1988) 477–482.

[28] S.L. Manley, K.D. Goodwin, W.J. North, Laboratory production of bromoform, methylene bromide, and methyl iodide by macroalgae and distribution in near-shore southern California waters, Limnol. Oceanogr. 37 (1992) 1652–1659.

[29] R.M. Moore, R. Tokarczyk, V.K. Tait, M. Poulin, C. Green, Marine phytoplankton as a natural source of volatile organohalogens, in: A. Grimvall, E.W.B. de Leer (Eds.), Naturally-Produced Organohalogens, Kluwer Academic Publishers, Dordrecht, 1995, pp. 283–294.

[30] P.D. Nightingale, G. Malin, P.S. Liss, Production of chloroform and other low-molecular-weight halocarbons by some species of macroalgae, Limnol. Oceanogr. 40 (1995) 680–689.

[31] S.L. Manley, J. de la Cuesta, Methyl iodide (CH_3I) production from marine phytoplankton cultures, Limnol. Oceanogr. 42 (1997) 142–147.

[32] S.L. Manley, P.E. Barbero, Physiological constraints on bromoform ($CHBr_3$) production by *Ulva lactuca* (Chlorophyta), Limnol. Oceanogr. 46 (2001) 1392–1399.

[33] H. Yamamoto, Y. Yokouchi, A. Otsuki, H. Itoh, Depth profiles of volatile halogenated hydrocarbons in seawater in the Bay of Bengal, Chemosphere 45 (2001) 371–377.

[34] F. Laturnus, T. Svensson, C. Wiencke, G. Öberg, Ultraviolet radiation affects emission of ozone-depleting substances by marine macroalgae: results from a laboratory incubation study, Environ. Sci. Technol. 38 (2004) 6605–6609.

[35] A.M. Wuosmaa, L.P. Hager, Methyl chloride transferase: a carbocation route for biosynthesis of halometabolites, Science 249 (1990) 160–162.

[36] R.M. Moore, M. Webb, R. Tokarczyk, R. Wever, Bromoperoxidase and iodoperoxidase enzymes and production of halogenated methanes in marine diatom cultures, J. Geophys. Res. 101 (1996) 20899–20908.

[37] S.L. Manley, Phytogenesis of halomethanes: a product of selection or a metabolic accident?, Biogeochem. 60 (2002) 163–180.

[38] R. Theiler, J.C. Cook, L.P. Hager, Halohydrocarbon synthesis of bromoperoxidase, Science 202 (1978) 1094–1096.

[39] P.S. Liss, The air-sea exchange of low molecular weight halocarbon gases, in: P. Buat-Ménard (Ed.), The role of Air-Sea gas Exchange in Geochemical Cycling, D. Reidel Pub. Co., Dordrecht, 1986, pp. 238–294.

[40] N. Bell, L. Hsu, D.J. Jacob, M.G. Schultz, D.R. Blake, J.M. Lobert, E. Maier-Reimer, Methyl iodide: atmospheric budget and use as a tracer of

marine convection in global models, J. Geophys. Res. 107 (2002) 4340–4352.

[41] S. Klick, K. Abrahamsson, Biogenic volatile iodated hydrocarbons in the ocean, J. Geophys. Res. 97 (1992) 12683–12687.

[42] R. Vogt, R. Sander, R. von Glasow, P.J. Crutzen, Iodine chemistry and its role in halogen activation and ozone loss in the marine boundary layer: a model study, J. Atmos. Chem. 32 (1999) 375–395.

[43] M. Mtolera, J. Collén, M. Pedersén, A. Ekdahl, K. Abrahamsson, A. Semesi, Stress induced production of volatile halogenated organic compounds in *Eucheuma denticulatum* (Rhodophyta) caused by elevated pH and high light intensity, Eur. J. Phycol. 31 (1996) 89–95.

[44] F. Laturnus, C. Wiencke, H. Klöser, Antarctic macroalgae—sources of volatile halogenated organic compounds, Mar. Environ. Res. 41 (1996) 169–181.

[45] B. Giese, F. Laturnus, F.C. Adams, C. Wiencke, Release of volatile C1–C4 by marine macroalgae from various climate zones, Environ. Sci. Technol. 33 (1999) 2432–2439.

[46] L.J. Carpenter, W.T. Sturges, P.S. Liss, S.A. Penkett, B. Alicke, K. Hebestreit, U. Platt, Short-lived alkyl iodides and bromides at Mace-Head, Ireland: links to biogenic sources and halogen oxide production, J. Geophys. Res. 104 (1999) 1679–1689.

[47] P. Dangeard, Sur le dégagement de l'iode chez les algues marines, C. R. Acad. Sc. Paris 186 (1928) 892–894.

[48] P. Dangeard, Recherches sur les iodures, l'iodovolatilisation et les oxydases, Le Botaniste 22 (1930) 33–73.

[49] H. Kylin, Über das Vorkommen von Jodiden, Bromiden und Jodidoxydasen bei Meeresalgen, Hoppe Seylers Z. Physiol. Chem. 186 (1929) 50–84.

[50] M. Pedersén, J. Collén, K. Abrahamsson, A. Ekdahl, Production of halocarbons from seaweeds: an oxidative stress reaction?, Sci. Mar. 60 (1996) 257–263.

[51] G. Malin, F.C. Küpper, L.J. Carpenter, A. Baker, W. Broadgate, B. Kloareg, P.S. Liss, Trace gas production by seaweeds: defense, oxidative stress, signalling and atmospheric significance, J. Phycol. 37 (2001) 32.

[52] K.J. Whittle, Marine organisms and their contribution to organic matter in the ocean, Mar. Chem. 5 (1977) 381–411.

[53] C.D. O'Dowd, J.L. Jimenez, R. Bahreini, R.C. Flagan, J.H. Seinfeld, K. Hämeri, L. Pirjola, M. Kulmala, G.S. Jennings, T. Hoffmann, Marine aerosol formation from biogenic iodine emissions, Nature 417 (2002) 632–636.

[54] G. McFiggans, H. Coe, R. Burgess, J. Allan, M. Cubison, M.R. Alfarra, R. Saunders, A. Saiz-Lopez, J.M.C. Plane, D. Wevill, L.J. Carpenter, A.R. Rickard, P.S. Monks, Direct evidence for coastal iodine particles from *Laminaria* macroalgae—linkage to emissions of molecular iodine, Atm. Chem. Phys. 4 (2004) 701–713.

[55] T. Hoffmann, C.D. O'Dowd, J.H. Seinfeld, Iodine oxide homogeneous nucleation: an explanation for coastal new particle production, Geophys. Res. Lett. 28 (2001) 1949–1952.

[56] G. McFiggans, J.M.C. Plane, B.J. Allan, L.J. Carpenter, H. Coe, C.D. O'Dowd, A modeling study of iodine chemistry in the marine boundary layer, J. Geophys. Res. 105 (2000) 14371–14386.

[57] A.R. Baker, Marine aerosol iodine chemistry: the importance of soluble organic iodine, Environ. Chem. 2 (2005) 295–298.

[58] Y. Muramatsu, S. Yoshida, U. Fehn, S. Amachi, Y. Ohmomo, Studies with natural and anthropogenic iodine isotopes: iodine distribution and cycling in the global environment, J. Environ. Rad. 74 (2004) 221–232.

[59] G.M. Raisbeck, F. Yiou, ^{129}I in the oceans: origins and applications, Sci. Total Environ. 237–238 (1999) 31–41.

[60] N. Buraglio, A. Aldahan, G. Possnert, I. Vintersved, ^{129}I from the nuclear reprocessing facilities traced in precipitation and runoff in northern Europe, Environ. Sci. Technol. 35 (2001) 1579–1586.

[61] P. Bailly du Bois, F. Dumas, Fast hydrodynamic model for medium- and long-term dispersion in seawater in the English Channel and southern North Sea, qualitative and quantitative validation by radionuclide tracers, Ocean Mod. 9 (2005) 169–210.

[62] M. Masson, P. Germain, B. Eyssaultier, A. Grauby, Évolution de la radioactivité de produits marins des côtes de la Manche au cours de la transformation agroalimentaire (algues) ou au cours de la préparation culinaire (moules), in: Radioactivity Transfer During Food Processing and Culinary Preparation, Commission of the European Communities Directorate General XI, Luxembourg, 1989, pp. 457–468.

[63] D. Maro, C. Rommens, D. Hébert, M. Masson, P. Bouisset, P. Germain, J. Loiseau, The transfer of artificial radioelements contained in seaweed to food transformation products (alginates and carrageenans), Radioprotection–Colloques 37 (61) (2002) 1323–1328.

[64] R.C. Wilson, J. Vives, I. Battle, P. McDonald, T.G. Parker, Uptake and depuration of ^{131}I by the edible periwinkle *Littorina littorea*: uptake from labelled seaweed (*Chondrus crispus*), J. Environ. Rad. 80 (2005) 259–271.

[65] G.N. Saenko, Y.Y. Kravtsova, V.V. Ivanenko, S.I. Sheludko, Concentration of iodine and bromine by plants in the Seas of Japan and Okhotsk, Mar. Biol. 47 (1978) 243–250.

[66] E. Ar Gall, F.C. Küpper, B. Kloareg, A survey of iodine contents in *Laminaria digitata*, Bot. Mar. 47 (2004) 30–37.

[67] M.A. Amat, Physiology of Iodine Uptake and Distribution in *Laminaria saccharina* (L.) Lamouroux, Simon Fraser University, Vancouver, Ph.D. thesis, 1985.

[68] M.A. Amat, L.M. Srivastava, Translocation of iodine in *Laminaria saccharina* (Phaeophyta), J. Phycol. 21 (1985) 330–333.

[69] J. Roche, Y. Yagi, Sur la fixation de l'iode radioactif par les algues et sur les constituants iodés des laminaires, C. R. Soc. Biol. Paris 146 (1952) 642–645.

[70] R. Scott, Observations on the iodo-amino-acids of marine algae using iodine-131, Nature 173 (1954) 1098–1099.

[71] T.I. Shaw, The mechanism of iodide accumulation by the brown sea weed *Laminaria digitata*. The uptake of ^{131}I, Proc. R. Soc. Lond. B151 (1959) 356–371.

[72] T.I. Shaw, The mechanism of iodide accumulation by the brown sea weed *Laminaria digitata*. II. Respiration and iodide uptake, Proc. R. Soc. Lond. B152 (1959) 109–117.

[73] M. Shah, R.G. Wuilloud, S.S. Kannamkumaratha, J.A. Caruso, Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection, J. Anal. At. Spectrom. 20 (2005) 176–182.

[74] M.C. Feiters, C. Leblanc, F.C. Küpper, W. Meyer-Klaucke, G. Michel, P. Potin, Bromine is an endogenous component of a vanadium bromoperoxidase, J. Am. Chem. Soc. 127 (2005) 15340–15341.

[75] P. Dangeard, L'iode des algues, les ioduques, l'iodovolatilisation, Le Botaniste 41 (1957) 187–207.

[76] V.W. Truesdale, I.G.W. Luther, C. Canosa-Mas, Molecular iodine reduction in seawater, an improved rate equation considering organic compounds, Mar. Chem. 48 (1995) 143.

[77] C. Colin, C. Leblanc, E. Wagner, L. Delage, E. Leize-Wagner, A. van Dorsselaer, B. Kloareg, P. Potin, The brown algal kelp *Laminaria digitata* features distinct bromoperoxidase and iodoperoxidase activities, J. Biol. Chem. 278 (2003) 23545–23552.

[78] C. Colin, C. Leblanc, G. Michel, E. Wagner, E. Leize-Wagner, A. van Dorsselaer, P. Potin, Vanadium-dependent iodoperoxidases in *Laminaria digitata*, a novel biochemical function diverging from brown algal bromoperoxidases, J. Biol. Inorg. Chem. 10 (2005) 156–166.

[79] P. Potin, K. Bouarab, J.-P. Salaün, G. Pohnert, B. Kloareg, Biotic interactions of marine algae, Curr. Opin. Plant Biol. 5 (2002) 308–317.

[80] H. Vilter, Peroxidases from phaeophyceae: a vanadium(V)-dependent peroxidase from *Ascophyllum nodosum*, Phytochem. 23 (1984) 1387–1390.

[81] H. Vilter, Vanadium-dependent haloperoxidases, in: H. Sigel, A. Sigel (Eds.), Metal Ions in Biological Systems, Marcel Dekker, Inc., New York, Basel, Hong Kong, 1995, pp. 325–362.

[82] P. Jordan, H. Vilter, Native bromoperoxidases do not bind to nitrocellulose: use of DEAE-cellulose as an alternative in blotting, Electrophoresis 11 (1990) 653–655.

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

[83] M. Almeida, S. Filipe, M. Humanes, M.F. Maia, R. Melo, N. Severino, J.A.L. da Silva, J.J.R. Frausto da Silva, R. Wever, Vanadium haloperoxidases from brown algae of the Laminariaceae family, Phytochem. 57 (2001) 633–642.

[84] H.S. Soedjak, A. Butler, Characterization of vanadium bromoperoxidase from *Macrocystis* and *Fucus*: reactivity of vanadium bromoperoxidase toward acyl and alkyl peroxides and bromination of amines, Biochem. 29 (1990) 7974–7981.

[85] H.S. Soedjak, A. Butler, Chlorination catalyzed by vanadium bromoperoxidase, Inorg. Chem. 29 (1990) 5015–5017.

[86] H. Vilter, Aqueous two-phase systems, Methods Enz. 228 (1994) 665–672.

[87] M.N. Isupov, A.R. Dalby, A.A. Brindley, Y. Izumi, T. Tanabe, G.N. Murshudov, J.A. Littlechild, Crystal structure of dodecameric vanadium-dependent bromoperoxidase from the red alga *Corallina officinalis*, J. Mol. Biol. 299 (2000) 1035–1049.

[88] J.A. Littlechild, E. Garcia-Rodriguez, Structural studies on the dodecameric vanadium bromoperoxidase from *Corallina* species, Coord. Chem. Rev. 237 (2003) 65–76.

[89] A. Butler, J.N. Carter, M.T. Simpson, Vanadium in proteins and enzymes, in: I. Bertini, A. Sigel, H. Sigel (Eds.), Handbook on Metalloproteins, Marcel Dekker, Inc., New York, Basel, 2001, pp. 153–179.

[90] J.A. Littlechild, Haloperoxidases and their role in biotransformation reactions, Curr. Opin. Chem. Biol. 3 (1999) 28–34.

[91] M. Weyand, H.J. Hecht, M. Kiess, M.-F. Liaud, H. Vilter, D. Schomburg, X-ray structure determination of a vanadium-dependent haloperoxidase from *Ascophyllum nodosum* at 2.0 Å resolution, J. Mol. Biol. 293 (1999) 595–611.

[92] V. Vreeland, K.L. Ng, L. Epstein, cDNA sequence and active recombinant vanadium bromoperoxidase from *Fucus* embryos, Mol. Biol. Cell 9 (1998) 1043.

[93] J.N. Carter, K.E. Beatty, M.T. Simpson, A. Butler, Reactivity of recombinant and mutant vanadium bromoperoxidase from the red alga *Corallina officinalis*, J. Inorg. Biochem. 91 (2002) 59–69.

[94] M. Shimonishi, S. Kuwamoto, H. Inoue, R. Wever, T. Ohshiro, Y. Izumi, T. Tanabe, Cloning and expression of the gene for a vanadium-dependent bromoperoxidase from a marine macro-alga, *Corallina pilulifera*, FEBS Lett. 428 (1998) 105–110.

[95] T. Ohshiro, W. Hemrika, T. Aibara, R. Wever, Y. Izumi, Expression of the vanadium-dependent bromoperoxidase gene from a marine macroalga *Corallina pilulifera* in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme, Phytochem. 60 (2002) 595–601.

[96] T. Ohshiro, J.A. Littlechild, E. Garcia-Rodriguez, M.N. Isupov, Y. Iida, T. Kobayashi, Y. Izumi, Modification of halogen specificity of a vanadium-dependent bromoperoxidase, Protein Sci. 13 (2004) 1566–1571.

[97] A. Messerschmidt, R. Wever, X-ray structure of a vanadium-containing enzyme: chloroperoxidase from the fungus *Curvularia inaequalis*, Proc. Natl. Acad. Sci. USA 93 (1996) 392–396.

[98] A. Messerschmidt, L. Prade, R. Wever, Implications for the catalytic mechanism of the vanadium-containing enzyme chloroperoxidase from the fungus *Curvularia inaequalis* by X-ray structures of the native and peroxide form, Biol. Chem. 378 (1997) 309–315.

[99] W. Hemrika, R. Renirie, S. Macedo-Ribeiro, A. Messerschmidt, R. Wever, Heterologous expression of the vanadium-containing chloroperoxidase from *Curvularia inaequalis* in *Saccharomyces cerevisiae* and site-directed mutagenesis of the active site residues His(496), Lys (353), Arg(360), and Arg(490), J. Biol. Chem. 274 (1999) 23820–23827.

[100] S. Macedo-Ribeiro, W. Hemrika, R. Renirie, R. Wever, A. Messerschmidt, X-ray crystal structures of active site mutants of the vanadium-containing chloroperoxidase from the fungus *Curvularia inaequalis*, J. Biol. Inorg. Chem. 4 (1999) 209–219.

[101] J.M. Arber, E. de Boer, C.D. Garner, S.S. Hasnain, R. Wever, Vanadium K-edge X-ray absorption spectroscopy of bromoperoxidase from *Ascophyllum nodosum*, Biochem. 28 (1989) 7968–7973.

[102] U. Küsthardt, B. Hedman, K.O. Hodgson, R. Hahn, H. Vilter, High-resolution XANES studies on vanadium-containing haloperoxidase: pH-dependence and substrate binding, FEBS Lett. 329 (1993) 5–8.

[103] M. Casny, D. Rehder, H. Schmidt, H. Vilter, V. Conte, A (17)O NMR study of peroxide binding to the active centre of bromoperoxidase from *Ascophyllum nodosum*, J. Inorg. Biochem. 80 (2000) 157–160.

[104] U. Christmann, H. Dau, M. Haumann, E. Kiss, P. Liebisch, D. Rehder, G. Santoni, C. Schulzke, Substrate binding to vanadate-dependent bromoperoxidase from *Ascophyllum nodosum*: a vanadium K-edge XAS approach, Dalton Trans., 2004 (2534–2540).

[105] R. Renirie, W. Hemrika, R. Wever, Peroxidase and phosphatase activity of active-site mutants of vanadium chloroperoxidase from the fungus *Curvularia inaequalis*. Implications for the catalytic mechanisms, J. Biol. Chem. 275 (2000) 11650–11657.

[106] N. Tanaka, Z. Hasan, R. Wever, Kinetic characterization of active site mutants Ser402Ala and Phe397His of vanadium chloroperoxidase from the fungus *Curvularia inaequalis*, Inorg. Chim. Acta 356 (2003) 288–296.

[107] M. Eigen, K. Kustin, The kinetics of halogen hydrolysis, J. Am. Chem. Soc. 84 (1962) 1355–1361.

[108] H.S. Soedjak, J.V. Walker, A. Butler, Inhibition and inactivation of vanadium bromoperoxidase by the substrate hydrogen peroxide and further mechanistic studies, Biochem. 34 (1995) 12689–12696.

[109] R.R. Everett, H.S. Soedjak, A. Butler, Mechanism of dioxygen formation catalyzed by vanadium bromoperoxidase. Steady state kinetic analysis and comparison to the mechanism of bromination, J. Biol. Chem. 265 (1990) 15671–15679.

[110] R.A. Tschirret-Guth, A. Butler, Evidence for organic substrate binding to vanadium bromoperoxidase, J. Am. Chem. Soc. 116 (1994) 411–412.

[111] J.S. Martinez, G.L. Carroll, R.A. Tschirret-Guth, G. Altenhoff, D.R. Little, A. Butler, On the regiospecificity of vanadium bromoperoxidase, J. Am. Chem. Soc. 123 (2001) 3289–3294.

[112] M.A. Andersson, A. Willetts, S.G. Allenmark, Asymmetric sulfoxidation catalyzed by a vanadium-containing bromoperoxidase, J. Org. Chem. 62 (1997) 8455–8458.

[113] H.B. ten Brink, H.E. Schoemaker, R. Wever, Sulfoxidation mechanism of vanadium bromoperoxidase from *Ascophyllum nodosum*, Eur. J. Biochem. 268 (2001) 132–138.

[114] T. Briggs, W. Rauscher, An oscillating iodine–clock, J. Chem. Educ. 50 (1973) 496.

[115] K. Kyoung-Ran, J.L. Dong, J.S. Kook, A simplified model for the Briggs–Rauscher reaction mechanism, J. Chem. Phys. 117 (2002) 2710–2717.

[116] W. Gottardi, Iodine and desinfection: theoritical study on mode of actio, efficiency, stability and analytical aspects in the aqueous system, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 151–157.

[117] H.A. Liebhafsky, L.S. Wu, Reactions involving hydrogen peroxide, iodine, and iodate ion. V. Introduction to the oscillatory decomposition of hydrogen peroxide, J. Am. Chem. Soc. 96 (1974) 7180–7187.

[118] I. Stefanic, K.-D. Asmus, M. Bonifacié, Quantification of iodide oxidation by trichloromethyl peroxyl radicals and $I^- + I^2 = I^{3-}$ equilibrium in alcool/water mixtures, Phys. Chem. Chem. Phys. 5 (2003) 2783–2789.

[119] F.S. Zhang, R.M. Lynden-Bell, Solvent-Induced Symmetry Breaking, Phys. Rev. Lett. 90 (2003) 185505.

[120] F.S. Zhang, R.M. Lynden-Bell, Solvent-induced symmetry breaking: varying solvent strength, Phys. Rev. E 71 (2005) 21502.

[121] L.P. Hager, D.R. Morris, F.S. Brown, H. Eberwein, Chloroperoxidase. II. Utilization of halogen anions, J. Biol. Chem. 241 (1966) 1769–1777.

[122] E. de Boer, H. Plat, M.G.M. Tromp, R. Wever, M.C.R. Franssen, H.C. van der Plas, E.M. Meijer, H.E. Schoemaker, Vanadium containing bromoperoxidase: an example of an oxidoreductase with high operational stability in aqueous and organic media, Biotechnol. Bioeng. 30 (1987) 607.

[123] M. Huwiler, H. Kohler, Pseudo-catalytic degradation of hydrogen peroxide in the lactoperoxidase/H_2O_2/iodide system, Eur. J. Biochem. 141 (1984) 69–74.

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.

[124] P. Vanýsek, Electrochemical series, in: D.R. Lide (Ed.), Hanbook of Chemistry and Physics, CRC Press, London, 1997–1998, pp. 20–25.

[125] C.E. Kolb, Iodine's air of importance, Nature 417 (2002) 597–598.

[126] R. Ortega, S. Bohic, R. Tucoulou, A. Somogyi, G. Devès, Microchemical element imaging of yeast and human cells using synchrotron X-ray microprobe with Kirkpatrick–Baez optics, Anal. Chem. 76 (2004) 309–314.

[127] A. Schwarz, K.G. Heumann, Two-dimensional on-line detection of brominated and iodinated volatile organic compounds by ECD and ICP-MS after GC separation, Anal. Bioanal. Chem. 374 (2002) 212–219.

[128] S.A. Borchardt, E.J. Allain, J.J. Michels, G.W. Stearns, R.F. Kelly, W.F. McCoy, Reaction of acylated homoserine lactone bacterial signaling molecules with oxidized halogen antimicrobials, Appl. Environ. Microbiol. 67 (2001) 3174–3179.

[129] M. Steinke, G. Malin, P.S. Liss, Trophic interactions in the sea: an ecological role for climate relevant volatiles, J. Phycol. 38 (2002) 630–638.

[130] V. Roeder, J. Collén, S. Rousvoal, E. Corre, C. Leblanc, C. Boyen, Identification of stress genes transcripts in *Laminaria digitata* (Phaeophyceae) protoplast cultures by expressed sequence tag analysis, J. Phycol. 41 (2005) 1227–1235.

[131] M. Berglin, L. Delage, P. Potin, H. Vilter, H. Elwing, Enzymatic cross-linking of a phenolic polymer extracted from the marine alga *Fucus serratus*, Biomacromolecules 5 (2004) 2376–2383.

Please cite this article as: C. Leblanc et al., Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases, Biochimie (2006), doi: 10.1016/j.biochi.2006.09.001.