KEY ASPECTS OF THE IODINE METABOLISM IN BROWN ALGAE: A BRIEF CRITICAL REVIEW

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KEY ASPECTS OF THE IODINE METABOLISM IN BROWN ALGAE: A BRIEF CRITICAL REVIEW

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Running Title: Iodine metabolism in *Brown Algae*

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Giant kelp (*Macrocystis pyrifera*, pictured here: in Port William, East Falkland, Feb. 2013) has a prolific halogen metabolism.

(Photo: F.C. Küpper)

Abstract

Brown algae include the strongest accumulators of iodine known among living systems. This paper reviews the current state of bioinorganic research in the field, focusing on the models *Laminaria digitata*, *Macrocystis pyrifera* and *Ectocarpus siliculosus*, and covering uptake and efflux, localization and biological significance of storage, as well as marine and atmospheric chemistry of iodine.

Significance to Metallomics

Although bioinorganic chemistry is often associated with biologically active metals, there are other inorganic elements that are important to life. Here we go down one of the less traveled paths of bioinorganic chemistry to focus on a halogen, iodine. Iodine has some metal-like character and it certainly is inorganic! While its essential role in human metabolism in the form of the thyroid hormones (such as thyroxine) is well known, fewer are aware of its extensive role in the metabolism of brown seaweeds, information of which is largely confined to the atmospheric and phycology literature. One of the basic hypotheses concerning the origins of life is that reactions based on, or catalyzed by, simple inorganic elements were likely precursors of the complex organic based biological processes characteristic of later life forms. Attack by pathogens and, after the great oxygenation event, oxidative stress, led to the evolution of forms of innate immunity and antioxidant capabilities such as those seen in modern plants and animals. It seems possible, if not probable, that these processes may have had a strictly inorganic beginning. The brown algae, which constitute a very old lineage, are perhaps unique in having forms of antioxidants and innate immunity that appear to be strictly inorganic and based on iodine. As such, the study of these inorganic based processes can possibly shed some light on their evolutionary history*.* Thus here we review the fascinating bioinorganic chemistry of iodine in this environmentally important group of marine algae.

Why Algae? Algae are a polyphyletic assemblage of autotrophic, plant-like organisms, which generally live in aquatic environments and lack the typical structure (roots-stem-leaves) and tissues of terrestrial plants. Marine algae are critically important plant-like members of the ocean community which make up most of global marine primary productivity and influence climate by controlling processes such as biogenic calcification, oceanic sequestration of CO_2 and release of dimethylsulfide [1]. In addition many microalgae (phytoplankton), including the climatically important diatoms, dinoflagellates and coccolithophores, can form blooms containing toxic or harmful species that have occurred with increasing frequency in recent decades, and have caused substantial ecological and economic damage worldwide. Likewise the macroalgae or seaweeds, brown algae in particular, can be the dominant organisms, in terms of biomass, of the marine coastal environment often forming extensive kelp forests.

Brown algae (Phaeophyta) belong to a lineage that has evolved independently of other major photosynthetic lineages, such as green plants (Chlorophyta) and red algae (Rhodophyta). Instead, they are classified within the Stramenopiles and Chromalveolates together with diatoms, golden-brown algae and oomycetes [2]. They also represent one of the few eukaryotic linages that have developed multicellularity. As a consequence of this singular evolutionary history, brown algae exhibit many unusual, and often unique, features. These features are adaptations to the potentially harsh marine coastal environments in which brown algae are often the dominant organisms in terms of biomass. The key role of kelp forests, effectively constituting an interface between the ocean, the atmosphere and land masses, in the biogeochemical cycle of halogens is well established [3]. Their role in marine benthic carbon sequestration is the subject of ongoing research [4] and there is concern about their regression or changes in keystone composition and ecosystem functioning in the context of climate change [5] The industrial exploitation of marine algae is also expanding due to interest in their use for production of phycocolloids such as agar and carrageenans, alginate, fucans etc., but also for their potential as biofuels where they have the advantage of high productivity without competing with terrestrial crops for farmland. The alginate industry already former supports an economic activity of several hundred million dollars annually. Finally it may be mentioned that many are also of major economic importance as an important foodstuff in Asia and particularly in Japan and Korea (nori, kombu, wakame).

While there are many marine macroalgal species that accumulate iodine here we focus our attention on three of the most widely studied brown algae that occupy different ecological niches and compare their iodine metabolism. *Ectocarpus siliculosus* is a filamentous brown alga which has a worldwide distribution along temperate coastlines and is a nuisance as a fouling organism on many man-made surfaces in the sea. It has many significant advantages as an experimental model since it can be readily cultured in the laboratory, many facets of its biology have been well studied [6] and it is the only brown alga whose genome has been sequenced [7]. While *Ectocarpus* is a small brown alga with only two cell types, *Macrocystis pyrifera* (giant kelp) is characterized by large size and complex morphology, including meristems that repeatedly split and the occurrence of gas-filled pneumatocysts that buoy the thallus at the sea surface and a variety of both tissue and cell types (meristoderm, hyphal, cortical, sieve etc.). *Macrocystis* dominates the coastal ecosystem from the Pacific coast of central Baja

California, México, to central California, USA, and parts of coastal Alaska. *Macrocystis* is also the dominant canopy forming kelp throughout much of the coastal ecosystems in the southern Hemisphere [8]. *Macrocystis* is of paramount ecological and economic importance as it is the single largest source of raw material for the global alginate industry. Finally *Laminaria digitata* (Oarweed), is the major kelp species on North Atlantic rocky shores, including Maine and the Canadian Maritimes, Newfoundland and the European Atlantic coast from Brittany (France) to northern Norway. It is the strongest iodine accumulator among all living systems that is currently known [9], and its iodine emissions have an established impact on aerosol formation [10-12] .

Environmental Aspects: Brown algae are unique in that they feature a highly evolved halogen metabolism. The genus *Laminaria* comprises the strongest accumulators of iodine currently known and are major emitters of both molecular iodine and iodinated organics into the atmosphere [11]. These latter species are important contributors to the surface destruction of tropospheric ozone, contributing to coastal cloud formation and are an important link between ocean biology, atmospheric composition, and climate. It was originally thought that biologically produced CH_3I and CH_2I_2 would be the most important atmospherically active volatile iodine containing species. However, it has been subsequently shown that in fact inorganic iodine emissions (I_2 and/or HOI) dominate by at least five orders of magnitude over the organoiodine species [3a, 10b]. Interestingly, the total number of mole of I_2 emitted by *L. digitata* stipes was approximately 10 times higher than those emitted from other thallus parts [12]. After exposure to air for between 60 and 180 min, I_2 emission rates of all thallus parts were reduced by 70-80% [12]. Chamber experiments with *L. digitata* demonstrated that emission of I_2 occurred in four distinct stages: (1) moderate emissions from partially submerged samples; (2) a strong release by fully emerged samples; (3) slowing or stopping of I_2 release; and (4) later pulses of I_2 evident in some samples [13]. Also, aerosol particle concentration produced from I_2 is more than a factor of 10 higher than that produced from CH_2I_2 for the same mixing ratios [14]. The emission of molecular iodine is more widespread in brown algae – also the fucoids *Fucus vesiculosus* and *Ascophyllum nodosum* have been found to be strong emitters [15]. Brown algae influence the concentration and speciation of iodine in coastal seawater. *Laminaria digitata* and *Fucus serratus* take up iodide under unstressed, steady-state conditions [9, 16]. Conversely, *Laminaria digitata*, *Fucus serratus* and the red alga *Kallymenia antarctica* have also been observed to release iodide when stressed [9, 16c, 17]. *Macrocystis pyrifera* forests have been found to influence iodine speciation in coastal seawater[18], and both living *L. digitata* beds as well as decaying biomass of this kelp species, on the seashore and in shallow coastal waters, depending on physiological circumstances, either take up release iodide into seawater and molecular iodine into the coastal atmosphere, respectively [19].

Biological Role: Innate Immunity. While the hyperaccumulation of iodine by brown algae has been known for some time – in fact, iodine as a novel chemical element was discovered in ashes of kelps and fucoids in the context of the Napoleonic wars [3b, 20] and that molecular iodine emissions were related to environmental stressors [10b], its exact biological function largely remained a mystery. However, following a hypothesis from the medical field [21], more recent work provided support for the notion that iodide accumulation serves the provision of a simple, inorganic antioxidant [3a]. This would be linked to the innate immune response following elicitor recognition in brown algae [22] which has been corroborated by a study showing that defense responses in *Laminaria* involve tightly regulated iodine metabolism [23]. Furthermore, a recent study also suggested that iodide accumulation contributes to osmoregulation in *Laminaria*, and that it positively affects photo-physiology [24].

Marine algae, like terrestrial plants, are invaded by an array of pathogens of which only a few succeed in causing disease. The attack by pathogens is countered by an innate immune system similar to the well described one(s) possessed by terrestrial plants. The relevant plant immune system is broadly defined as microbial-associated molecular-patterns-triggered immunity (MTI). MTI involves the recognition of conserved, microbial elicitors called microbial-associated molecular patterns (MAMPs) by a class of plasma-membrane-bound extracellular receptors called pattern recognition receptors (PRRs) [25]. Many plant pathogens produce lytic enzymes to breach the structural barriers of plant tissues. The products, such as cell wall fragments, which are generated as a consequence, can function as endogenous elicitors called damage-associated molecular patterns (DAMPs). These DAMPs characteristically emerge in the apoplast and serve as danger signals to induce innate immunity similar to MAMPs [26]. The surface receptors (PRRs) which detect MAMPs include receptor-like kinases (RLK) and receptor-like proteins (RLP) [14].

The initial defense response elicited by plant cell cultures in response to MAMPs is the alkalinization of the growth medium. Occurring 0.5 to 2 min after elicitation, this event relies on drastic changes in fluxes of H^+, K^+, and Ca^{2+} ions across the plasma membrane [27]. The production of ROS at the cell surface, known as the oxidative burst, is also one of the earliest detectable events of plant defense. The ROS synthesis pathway has been deciphered commonly starting with an NAD(P)H oxidase reducing molecular oxygen to superoxide, which is subsequently converted by superoxide dismutase (SOD) to H_2O_2. ROS are potentially toxic analogous of reduced oxygen forms, such as the superoxide anion and hydrogen peroxide. They are considered to exert a direct antimicrobial action and contribute to a strengthening of the cell wall through oxidative cross linking of glycoproteins.

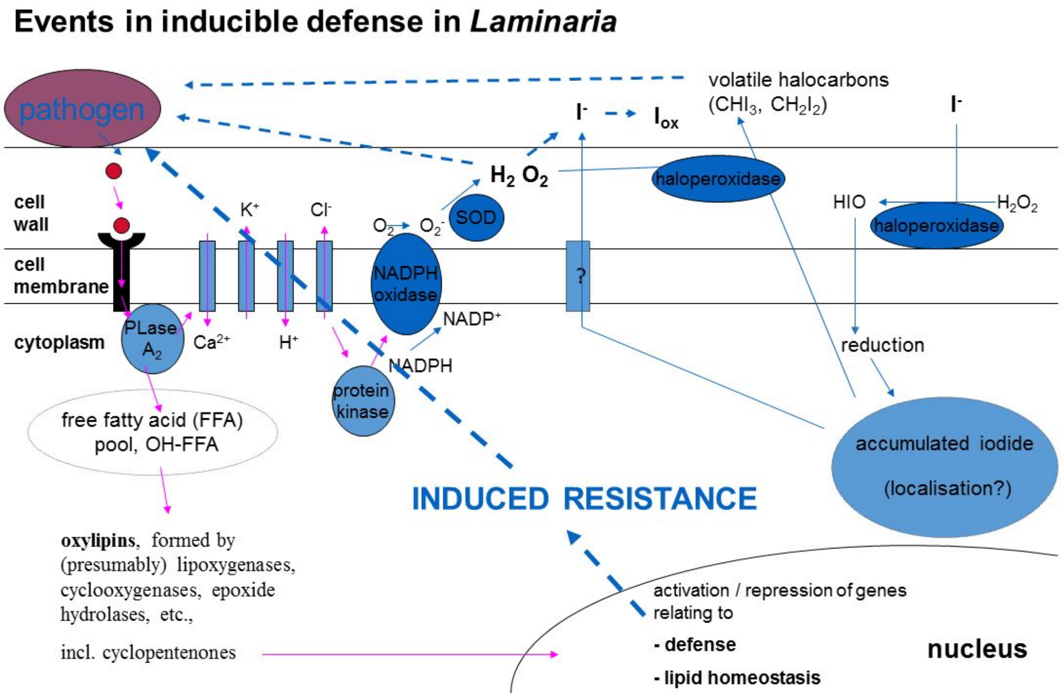

Figure 1: Proposed events in the inducible innate immune response of *Laminaria*.

Many of the above events characteristic of plant defense reactions appear to be conserved in the evolutionarily distant brown algae (figure 1). Upon recognition of elicitors (MAMPS or DAMPs), which can be either endogenous such as oligoalginates [22] or exogenous such as lipopolysaccharides from bacterial cell walls [28], *Laminaria* cells respond with an oxidative burst of hydrogen peroxide followed shortly thereafter by a massive efflux of iodide. Analogous to the formation of HOCl and HOBr by peroxidases in eosinophils[29], this in turn leads to formation of the highly bactericidal HOI and/or I_2 species (Eqn 1,2) in the apoplast serving as an antibacterial defense mechanism.

$$I^{-} + H_2O_2 \leftrightarrows HOI + OH^{-} \qquad (1)$$

$$HIO + I^{-} + H^{+} \leftrightarrows I_2 + H_2O \qquad (2)$$

Biological Role: Oxidative Stress Response. An alternate role/additional role for iodide is as an antioxidant as part of an oxidative stress response. For example, at low tide *Laminaria* thalli are exposed to high light levels, atmospheric ozone and desiccation producing extreme oxidative stress. High concentrations of iodide on the wet kelp surface detoxify ground-level ozone, resulting in the formation of iodine oxides and, if light is present, particles, leading to aerosol formation [3a, 10b] Based on this, it was proposed that iodide acts as an inorganic, simple antioxidant protecting the apoplast and thallus surface against oxidative stress [3a] It compares well with the established organic biological antioxidants in its reactivity with hydrogen peroxide, superoxide, ozone and hydroxyl radicals in both thermodynamics and kinetics but it is complemented in *Laminaria* by bromide for detoxifying superoxide [30]. We have also found that the total amount of iodine sequestered in samples of *Macrocystis* appeared to be depth-dependent with samples taken from several meters in depth having statistically significantly less iodine than samples obtained from the surface. This observation is consistent with the hypothesis that iodine stores represent an oxidative stress response since cells exposed to the atmosphere at the surface are exposed to greater sunlight and oxidative stress and such cells should have higher iodine stores than cells that came from submerged sections of the kelp. It is also consistent with the general observation that *Ectocarpus* which is largely benthic or epiphytic and submerged at all times has the lowest iodine accumulation while *Laminaria* which is regularly subjected to desiccation and the atmosphere during low tides has the most.

Central to the functioning of iodide as an antioxidant is its strong efflux to scavenge excess hydrogen peroxide, HOI and other ROS catalyzed by vanadium haloperoxidase enzymes [3a]. Thus In the absence of a nucleophilic acceptor, excess HOI (eqn 1) reacts with peroxide to regenerate iodide as in eqn 3:

$$HOI + H_2O_2 \leftrightarrows O_2 + H_3O^+ + I^- \qquad (3)$$

Together reactions 1 and 3 lead to the overall iodide assisted, vanadium haloperoxidase catalyzed, disproportionation of two moles of peroxide into oxygen and water i.e. eqn 4:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \qquad (4)$$

Alternatively excess HOI can be removed by iodide as in eqn 2. Interestingly, an upregulation of the expression of the single haloperoxidase gene was recently demonstrated in *Ectocarpus* upon infection by the oomycete *Eurychasma dicksonii* [31].

Uptake: Kinetics and Mechanism. Brown algae are known to accumulate iodine from seawater to a remarkable degree with internal levels ranging from 0.05-5% dry weight which constitutes an increase from the concentrations in seawater (near 500 nM) of up to 10^5! However there is considerable variation in iodine content between different systematic groups, location, season, tissue type and life cycle [9a, 32].

It is clear that to achieve internal iodine concentrations that high against such a large concentration gradient that some sort of active transport or trapping mechanism rather than simple diffusion would be required. Indeed in all the cases examined thus far, iodine appears to be taken up as iodide and uptake follows saturation Michaelis-Menten like kinetics with "K_M" values in the µM range (~50 µM for *Laminaria digitata*, ~20 µM for *Macrocystis pyrifera* and ~3 µM for *Fucus vesiculosus*). It is harder to compare "V_{max}" values due to the different ways these numbers have been expressed by different authors but they are typically around 10±10 nmol/min/g FW. It should be noted however that given seawater iodine concentrations (*vide supra*) are in the nM rather than the µM range, none of these uptake systems are operating anywhere near saturation. In addition while most of the uptake experiments have assumed and utilized iodide as the active substrate, there is some evidence that iodate can also be taken up [16c, 18a] However given that iodide is the natural substrate of the algal uptake system, early observations suggested that iodide could undergo oxidation at brown algal surfaces to produce I_2 or HOI which are far more lipophilic than iodide. It was subsequently shown that this oxidation likely occurs via hydrogen peroxide catalyzed by vanadium-dependent haloperoxidases (VPO). Vanadium haloperoxidases, which have been covered in detail by a number of excellent reviews[33], are thus key enzymes for brown algal halogen metabolism, both for iodine accumulation [9b], antioxidant defense [3a] and halogenation reactions [34]. *Laminaria* contains a multigenic family of these enzymes, with two subfamilies – the bromo – and iodoperoxidases, respectively, which are hypothesized to fulfil the different, aforementioned functions [35]. A colorimetric assay developed in the context of studies on *Laminaria* enables steady-state analyses of iodoand bromoperoxidase activities [36]. It should be noted that the *Ectocarpus siliculosus* genome [7] and a transcriptome analysis of *Macrocystis pyrifera* [37] appear to contain only a single VPO (of unknown specificity), although another study of *Macrocystis* did suggest the presence of a multigenic family of VPOs similar to that seen in *Laminaria* [18b]. Some of the best evidence for the importance of VPOs in the actual cellular uptake of iodine comes from their total absence in *Laminaria* gametophytes which in turn do not appear to take up iodine from solution and its presence in sporophytes which do [9b]. However an potential alternate pathway for oxidation of iodide leading up to its transport in one of its oxidized forms exists which involves an interaction between Fe(III) and I- as shown in eqn 5. Such a reaction between iodide and iron has recently been proposed to occur in a marine haptophyte and in terrestrial soils[38].

$$\mathrm{Fe(III)} + 2\mathrm{I}^- \rightarrow 2\,\mathrm{Fe(II)} + \mathrm{I}_2 \qquad E^o = +0.24\mathrm{v} \qquad (5)$$

Following oxidation of iodide to HOI/I_2 by peroxide and VPO or Fe(III) it is proposed that these more lipophilic species are the ones transported across the cell membrane, or more likely given the reactive nature of these species, that they iodinate polyunsaturated fatty acids (PUFAs) of the cell membrane. Once inside the cell (*or apoplast)* either the iodine or the iodinated fatty acids are presumed to be rereduced back to iodide by unknown cellular reducing agents (cysteine/glutathione?) thereby trapping this charged species (known to be the stored form of iodide) inside the cell or apoplast and allowing an internal buildup of against a concentration gradient (figure 2). While the uptake mechanisms proposed in figure 2 is intellectually attractive, it is difficult to establish the identity of the actually transported species (i.e. I_2, HOI, I^-, IO_3^- iodinated PUFA etc.) and whether VPOs are actually involved. Thus controversy exists as to the nature of the primary form of iodine taken up by brown algal cells. The general consensus has for a long time been that iodide is the only form of iodine that is taken up. However some recent reports from both field and laboratory studies suggest that iodate may also be taken up **[16c].** While X-ray absorption spectroscopy has unambiguously shown that the bulk (> 99%) of the accumulated species is in the form of iodide[3a], there are reports of minor amounts of organoiodine species being present in *Laminaria*. The only review of iodinated natural products [39] – of which 110 were known as of 2006 - lists none from *L. digitata*. An early report [40] claimed that most of the iodine in *Laminaria* was organically bound, but provided no further information about its speciation. Another report [41] from the early era of iodine explorations in seaweeds showed that most iodine in *Laminaria* is in the form of iodide, with a minor proportion bound to unspecified organic compounds. More recently, XAS enabled detection of the artefactual formation of iodotyrosine in freeze-dried, re-hydrated *Laminaria* tissues [3a].

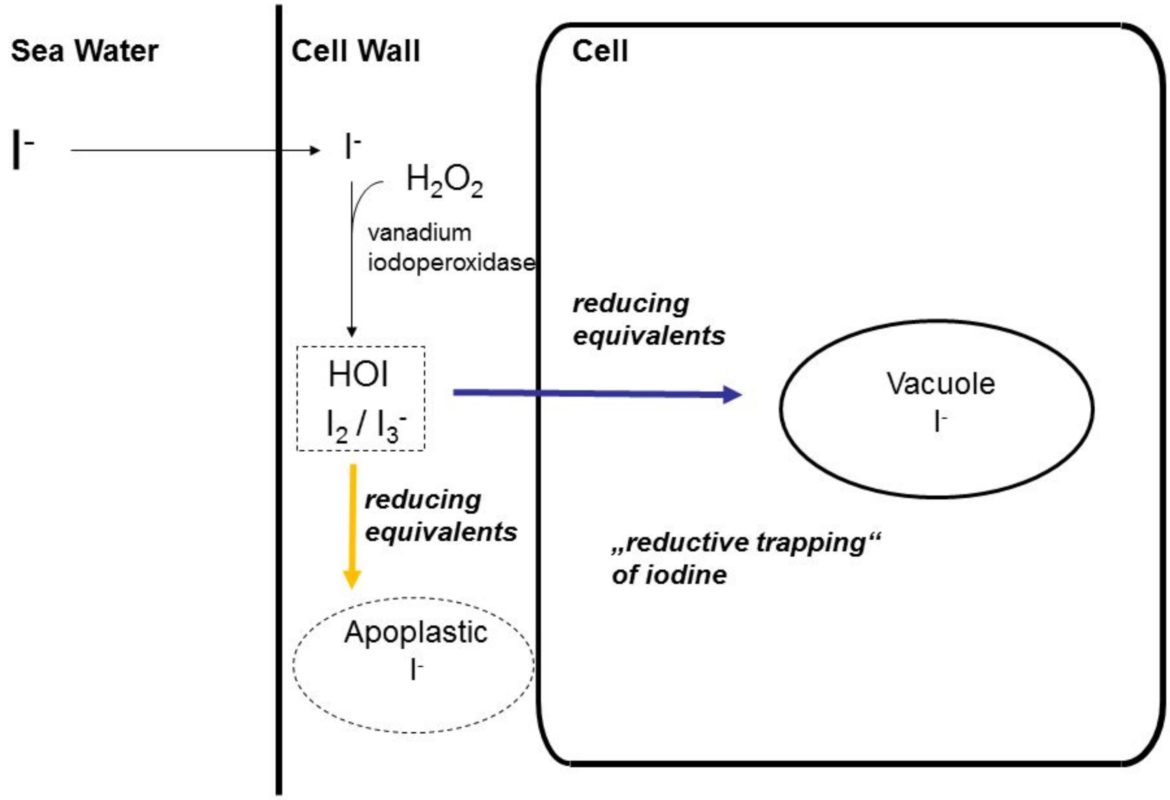

Figure 2: The two alternate pathways of iodine accumulation and storage in *Laminaria,* which upcoming research will need to resolve: Intracellular/vacuolar storage (blue arrow), based on the model proposed by Küpper *et al.* [9b] vs. apoplastic storage (yellow arrow), based on the model proposed by Verhaeghe *et al.* [42].

Storage: Localization and Mechanism. Central to the question of the mechanism of uptake is the question of where this process take place, and how, where and in what form is the iodine is stored for whatever biological use. Several lines of evidence including modern synchrotron-based methods such as EXAFS and XANES strongly indicate that the iodine that is internalized is almost entirely present as iodide (90+%) with much smaller amounts (if any) in the form of organoiodine compounds such as iodinated tyrosine etc. and/or iodate [3a, 32]. Indeed, XANES and EXAFS have been developed as a useful, non-invasive tool for probing the chemical speciation of iodine and bromine in seaweeds [3a, 30, 43]. EXAFS also enabled the detection of auto-halogenation by VPO. The original mechanism proposed by Küpper et al. has the iodide undergoing VPO-catalyzed oxidation at the cell wall with the actual product transported across the cell membrane (either directly, or via iodination of unsaturated fatty acids) followed by rereduction and storage as iodide within cells [9b]. At the tissue level there is general agreement that most (indeed almost all) of the iodine is located in and/or around the meristoderm (the peripheral layer of photosynthetically active cells) with concentrations dropping off dramatically progressing through the cortical cells into the central medulla. However at the single cell or subcellular level the localization of iodine remains controversial. Initial experiments suggested that iodine was stored internally in vacuoles [9b] but subsequent work using a variety of high level microchemical imaging methods reached the opposite conclusion i.e. that iodine was not stored internally but rather in the external apoplastic (cell wall) spaces and/or mucus layers [42]. Indeed, the localization of accumulated iodide – intracellular/vacuolar vs. apoplastic/cell wall-bound is one of the major open questions in this context. The differing results reported in the literature may well emanate from differences in sample preparation as the possibility for artifacts arising from elemental redistribution during preparation is always a concern especially in plant cells that have large vacuoles without internal structure so that movements of solutes during fixation are particularly likely. Thus most recently we conducted a 2D tomography study at the iodine Kα edge (thereby eliminating any calcium interference) of flash frozen and hydrated *Laminaria* stipe and blade at high resolution (100 nm) under cryogenic temperatures, conditions hat preclude any element redistribution. These preliminary data strongly suggest that unlike Ca and Sr, which are clearly located in the apoplastic/cell wall spaces, that iodine is NOT but rather is located internally in vacuoles of the meristoderm cells. If confirmed this will have a major impact on our understanding the mechanism of iodine uptake and efflux.

Once internalized, how iodide is held/fixed in the brown algal cells until required for efflux as an antioxidant response remains largely unknown. Such storage and release may be accomplished either via the presence of tertiary nitrogen groups which can be protonated or deprotonated as a function of pH or alternatively might involve binding through multivalent metal ions.

Efflux Kinetics and Mechanism. A variety of previous studies suggest that one of the function of the stored iodide in brown algae is as an inorganic antioxidant [3a]. In the presence of oxidative stress induced by hydrogen peroxide brown algae respond with an efflux of stored iodine [3a, 9, 17]. For example adult *Macrocystis* incubated in the presence of greater than 1 mM H_2O_2 released iodide into the surrounding seawater at an average rate of 0.50 ± 0.03 µmol/hr/g fresh weight. However iodide efflux was not linear but rather occurred as a burst beginning approximately 90-120 minutes post treatment. At the maximum H_2O_2 concentration tested, 2000 µM, kelp tissue released 0.49 µmoles of iodide / gram tissue. The same antioxidant response is seen in *Laminaria* [3a, 30]. However the response kinetics of iodine efflux following elicitor challenge is quite different: thus while efflux starts after a very short lag after elicitation in *Laminaria*, there is an approx. 1 hour lag phase in *Macrocystis*. At present, it is unclear what the underlying physiological or chemical cause is for the markedly different response kinetics.

A potentially attractive efflux mechanism (Figure 3) is provided by the observation that transport of iodide across artificial membranes is greatly enhanced by the presence of I_2 [44]. The data strongly supports a process in which membrane-localized I_2 acts as a carrier for I^- via the formation of I_3^- in the membrane as in eqn 6

$$I_2 + I^- \leftrightarrows I_3^- \qquad (6)$$

In this model as applied to algal iodide uptake or efflux the carrier, I_2, in the membrane is formed via the VPO catalyzed reaction of I^- with H_2O_2 as in eqns 1 and 2. This facilitated diffusion model is also consistent with the saturation kinetics seen. However given a measured equilibrium constant for eqn 6 of around 146 l/mole the rate of transport is highly dependent on the iodide concentration and it is clear that it cannot function as an uptake mechanism against a strong concentration gradient without some way of trapping any I^- delivered via dissociation of the I_3^- at the membrane interface.

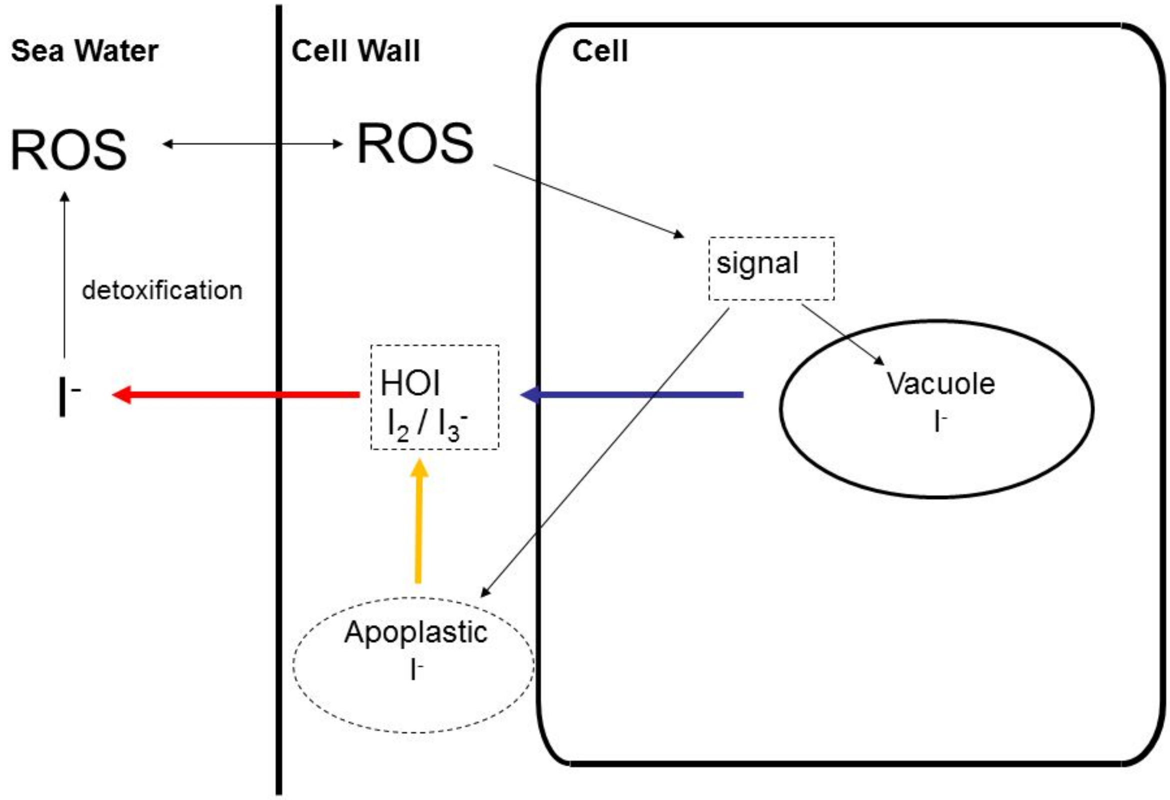

Figure 3: Under oxidative stress conditions, reactive oxygen species (ROS) lead to a release of iodide from either the vacuolar or apoplastic store. There is a transient formation of oxidized iodine species, however with most oxidants iodide is quickly regenerated. If the ROS is ozone, this leads to the formation of volatile I_2, impacting atmospheric processes.

<u>Conclusions and Future Directions</u><u>:</u> While much progress has been made in understanding iodine metabolism in brown algae since the discovery of the element in seaweed over 100 years ago, important questions remain to be addressed. These include:

a) Exactly where are iodine, iron and vanadium (surrogate for the VPO enzymes) localized in the meristoderm cells – and is there a functional link between them?
b) What is the exact mechanism for uptake and storage of iodine against a large concentration gradient?
c) How is stored iodide remobilized for efflux following a chemical or biological challenge?
d) What are the signaling pathways that result in both an oxidative burst following challenge with bacterial pathogens and the antioxidant response leading to efflux of iodide?
e) Besides elemental iodine emission, what metabolic processes result in the production and emission of the so-called very short lived organohalide species (i.e. simple halogenated organics such as CH_3I, CH_2I_2, etc.) and finally
f) Are similar processes in play for the other physiologically important halide, bromide?

Hopefully the answers to these and other as yet unthought-of questions will be discovered in the next few years as further studies on this fascinating bioinorganic system continue.

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