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Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide

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Abstract. Sporophytes of *Laminaria digitata* (L.) Lamour. were assayed for their content of accumulated iodine, which ranged from 0.4% of dry weight in adult plants up to 4.7% for young plantlets. Sporophyte tissue from *Laminaria saccharina* (L.) Lamour. and *L. digitata* took up iodide according to Michaelis-Menten kinetics. Hydrogen peroxide and various substances known to interfere with oxidative metabolism were shown to either inhibit or enhance the uptake of iodide, confirming that apoplastic oxidations play a key role in iodide uptake in *Laminaria*. Consistently, iodide uptake was triggered in *L. saccharina* protoplasts by incubation in the presence of hydrogen peroxide. Similarly, the uptake of iodide was enhanced in *L. digitata* gametophytes by addition of haloperoxidase, suggesting that this enzyme catalyses the oxidation of iodide by hydrogen peroxide and plays a key role in iodine uptake. Oxidative stress resulted in a marked efflux of the intracellular iodine. In both influx and efflux experiments, a marked proportion (10–30%) of the tracer was not accounted for, indicating volatilisation of iodine. The mechanism and possible functions of the accumulation of iodine by kelps are discussed.

Key words: Apoplastic oxidative metabolism – Haloperoxidase – Hydrogen peroxide – Iodine – *Laminaria*

Introduction

The total iodine concentration in the open ocean ranges between 0.44 and 0.49 μM. The major iodine species in coastal sea waters are iodate (IO_3^-) and iodide (I^-), along with smaller fractions of molecular iodine (I_2), hypoiodous acid (HIO) as well as various iodinated organic compounds (Truesdale et al. 1995). In the euphotic layer, iodide concentrations can reach as much as 50% of the total inorganic iodine, ca. 0.25 μM (Wong 1977). Brown algae from the order Laminariales accumulate iodine to more than 30,000 times the concentration of this element in seawater, up to levels as high as 1% of dry weight in *Laminaria digitata*, depending on wave exposure, geographic location, and depth (Black 1948, Young and Langille 1958; Saenko et al. 1978). Iodine was actually discovered in kelps by Courtois (see Shaw 1962) and Laminariales still remain a major source for the recovery of this element, mainly from the mariculture of *Laminaria japonica* in China (Wu and Lin 1987).

Understanding the biochemical pathways of iodine uptake in Laminariales has evoked interest since as early as the beginning of the twentieth century. Dangeard (1928) and Kylin (1929) first established the link between oxidative metabolism and the uptake of iodine. They discovered that the natural release of free iodine (I_2) from kelps, a process termed "iodovolatilisation", requires oxygen and takes place in the apoplast of the thallus surface cells. Since then, iodovolatilisation has been considered to account for the higher iodine content of the air in coastal areas (Dangeard 1957). Kylin (1929) postulated the involvement of an "iodide oxidase" in the thallus surface mucilage and discussed the oxidation of iodide prior to uptake, suggesting that I_2 was the species finally taken up. He presumed that, inside the cells, I_2 (0) would again be reduced to I^- (−1). Given the parallelism between iodine uptake kinetics by *L. digitata* and the speciation between molecular iodine and its aqueous form, Shaw (1959) proposed that HIO (+1) is the actual iodine form taken up in Laminariales.

The availability of radioactive ^{131}I allowed for accurate quantitative measurements of iodine uptake in brown algae (Roche and Yagi 1952). Uptake mechanisms were studied in the Fucales *Ascophyllum nodosum* (Baily and Kelly 1955) and *Fucus ceranoides* (Klemperer 1957), showing Michaelis-Menten kinetics and indicating that iodide and not iodate was the iodine

Abbreviations: AOS = activated oxygen species; DNP = 2,4-dinitrophenol; GOD = glucose oxidase; VHCs = volatile halogenated compounds

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species involved in the uptake. Based on the inhibition of iodine transport by various reducing agents in the kelp *Nereocystis luetkeana*, Tong and Chaikoff (1955) confirmed the dependence of iodine accumulation on oxygen and suggested involvement of hydrogen peroxide H_2O_2 in the oxidation of iodide. The radioactive tracer was detected in the tissue as inorganic iodine and mono- and di-iodotyrosine. These findings were later confirmed in *L. flexicaulis* (now *L. digitata*) by André (1965, 1971). No kinetic data are available for the accumulation of iodine in kelps, however, in spite of their marked ability to take up and concentrate iodine.

The presence of an alginate-bound peroxidase in *L. digitata* was reported by Murphy and O hEocha (1973b), who suggested its involvement in the oxidation of iodide as well as in the formation of iodoamino acids. More recently, non-heme vanadium (V)-containing haloperoxidases were discovered in the cell walls of brown algae (Vilter et al. 1983; Vilter 1984), including in the Laminariales (e.g., de Boer et al. 1986; Butler et al. 1990; Jordan and Vilter 1991). Haloperoxidases catalyse the oxidation of bromide and iodide into hypohalides. It was therefore suggested that, as catalysts of the oxidation of iodide into hypoiodous acid, haloperoxidases are involved in the accumulation of iodide in brown algae (Murphy and O hEocha 1973a; Vilter et al. 1983). However, no experimental evidence supporting involvement of haloperoxidase in iodine uptake has been reported so far.

In this paper, we report the kinetics of iodine accumulation by sporophytic tissues of *L. saccharina* and *L. digitata* and show that iodide is taken up according to a facilitated diffusion mechanism based on the oxidation of iodide in the apoplast. In addition, we demonstrate that the uptake of iodide by *L. digitata* gametophytes is markedly enhanced by incubation in the presence of haloperoxidase.

Materials and methods

Plant material and protoplast isolation. *Laminaria saccharina* (L.) Lamour and *Laminaria digitata* (L.) Lamour sporophytes were collected in the vicinity of Roscoff (Brittany, France) between October 1994 and July 1996. Sporophytes were kept in tanks of running sea water and used within 2 d after collection. For the experiments involving direct assessment of sporophyte tissue, discs, 1.7 cm in diameter, were punched out of the lower blade margins and allowed to recover in natural seawater for about 1 h. The discs averaged 58 mg and 220 mg in fresh weight for *L. saccharina* and *L. digitata*, respectively. Gametophyte cultures of *L. digitata* were established and maintained as described previously (Ar Gall et al. 1996).

Protoplasts were isolated from the meristematic regions and lower margins of *L. saccharina* blades, according to Benet et al. (1997). Yields ranged between 3×10^7 and 5×10^7 protoplasts · (g FW)$^{-1}$. Protoplasts were resuspended in artificial sea water (ASW) brought to an osmotic pressure of 1250 mosmol with KCl and $MgCl_2$ (ASW_{1250}: 400 mM NaCl, 80 mM $MgCl_2$, 22 mM $MgSO_4$, 85 mM KCl, 7 mM $CaCl_2$, 10 mM Hepes, pH adjusted to 8.2 with NaOH). Cell density was adjusted to 5×10^6 cells · ml^{-1} and the suspension was stored overnight in the dark at 12 °C on a slowly revolving rotary shaker. Iodine-uptake experiments were carried out within 24 h of protoplast isolation. Depending on the conditions of the experiment, cell densities were adjusted with ASW_{1250} to 2.5×10^6–2.5×10^5 cells · ml^{-1}. A control experiment showed that the use of ASW_{1250} instead of natural seawater resulted in no differences in iodine uptake.

Determination of accumulated iodine in L. digitata. *L. digitata* sporophytes were sampled in the sublittoral off Enez Glas near Roscoff and assayed for accumulated iodine as follows. Tissue disks were punched out of the blade with a cork borer, dried extensively at 60 °C and powdered with a Waring blender. Total iodine content was then determined by neutron activation analysis at the Département d'Analyse Élémentaire, Service Central d'Analyse, Centre National de la Recherche Scientifique (Vernaison, France).

Measurements of iodine uptake in Laminaria tissue and protoplasts. Tissue discs, gametophytes, or protoplasts were incubated at 10–15 °C on a rotary shaker in the presence of autoclaved, natural or artificial seawater, supplemented with various concentrations of KI. The amount of iodide contributed by the various salts in ASW was estimated at ca. 2 μM from the information from the suppliers regarding the contaminant trace elements. $Na^{123}I$ (supplied by CIS Bio International, Gif-sur-Yvette, France) or $Na^{125}I$ (supplied by Amersham, UK) was added to the incubation medium, resulting in a specific activity of 15 kBq · ml^{-1}(^{123}I) or 940 Bq · ml^{-1}(^{125}I), respectively, and with no significant change in iodide concentration. Biomass was designed so that depletion of radioactive iodine from the incubation medium at the end of the experiments was less than 20% of the initial radioactivity. At the end of incubation, 1-ml aliquots of the supernatant liquids were taken and tissue discs were rinsed exhaustively in seawater and blotted dry on a paper towel. The radioactivity present in the liquid and discs was determined with a gamma counter, consisting of a 7.6 × 7.6 cm well type NaI (Tl) crystal (Bicron), connected to a multichannel analyser (Canberra, France). Unless mentioned otherwise, measurements were performed in five independent replicates. The proportion of volatilised iodine was estimated by subtracting from the initial supply both the amount taken up by the tissue and that remaining in the incubation medium.

Protoplast or gametophyte suspensions were centrifuged for 3 min at 1,500 rpm using a Jouan GR 4.11 centrifuge, washed in ASW_{1250}, pelleted again and the radioactivity remaining in the pellet was determined by gamma counting. When using ^{123}I, data were corrected for the loss of tracer activity during the time course of experiments. Carry-over into protoplasts from the incubation medium was estimated in a series of control experiments, using [^{14}C] sorbitol (Amersham). The carry-over of radioactivity after 1 h incubation and one wash cycle was less than 0.6% of the tracer present in the original incubation medium and did not change significantly upon incubation in the presence of H_2O_2 or glucose oxidase (GOD)-glucose, whilst iodine accumulation in comparable protoplast suspensions amounted to about 10% of the initial supply within 10 min.

Monitoring of iodine efflux by L. digitata plantlets. *Laminaria digitata* plantlets, ca. 5 cm in size and with an average iodine content of 4.7% of dry weight (as described below), were charged for 24 h with ca. 37 kBq of iodine each (in volumes of 200 ml seawater). The radioactive incubation medium was then removed and plantlets were rinsed and left in 200 ml seawater for another 12–15 h, allowing for the determination under normal conditions of the release of accumulated iodine. Plantlets were finally transferred into 5 ml seawater and iodine efflux upon addition of 50 μM, 200 μM or 2 mM exogenous H_2O_2 was monitored by taking 400-μl aliquots at intervals of 5, 10, 15, 30, 60, 120 and 180 min after the onset of the stress. Measurements of the radioactivity remaining in the plantlets were also carried out, in order to compare the total amount of iodine released from the sporophytes with that recovered in the incubation medium.

Regulation of iodine uptake by peroxide and haloperoxidase. For studying the effect of H_2O_2 on iodine uptake, *Laminaria* samples were either directly provided with dilutions of a H_2O_2 stock solution

(Merck) or incubated in the presence of GOD (Sigma) and 100 mM glucose. Addition of 100 mM glucose alone did not result in any stimulatory effect on the uptake of iodine. The actual amount of H_2O_2 generated with the GOD-glucose system was assayed in seawater using the luminol chemiluminescence method (Glazener et al. 1991) and a Berthold LB 9507 luminometer. Hydrogen peroxide concentrations after 10 min were 63, 200, 910 μM for GOD activities of 0.025, 0.1 and 1.0 to 2.5 $U \cdot ml^{-1}$, respectively. Steady-state, permanent concentrations of 5 μM H_2O_2 were generated in *L. digitata* gametophyte suspensions by using 0.025 $U \cdot ml^{-1}$ GOD, 100 mM glucose and 6.4 $U \cdot ml^{-1}$ catalase.

A variety of chemical compounds known to interfere with oxidative metabolism was tested for possible inhibitory or enhancing effects on iodine uptake in tissue (see Table 1 for details). Unless mentioned otherwise, they were added to the tissue pieces in natural seawater 1 h prior to incubation with the radioactive tracer. The putative inhibitory effect on iodine uptake of 2,4-dinitrophenol (DNP; Amat, 1985) was investigated by preincubating protoplasts and tissue discs for 15, 30 and 60 min, with uncoupler concentrations ranging from 10 μM to 1 mM.

To address the possible involvement of haloperoxidase in iodine uptake, sporophyte tissue discs proved an unsuitable experimental system because of their high, yet heterogenous intrinsic rates of iodine uptake. *Laminaria digitata* gametophytes were therefore assayed for uptake of iodide in the presence of either or both H_2O_2 and haloperoxidase. Two different vanadium-(V)-dependent haloperoxidases were tested: a partially purified enzyme from *Ascophyllum nodosum*, referred to as A.n. I, a cell wall isoform from the transitional region between cortex and medulla (Vilter 1994); and the cortical isoenzyme from *L. digitata* (Jordan et al. 1991), prepared from freshly collected fronds according to Vilter (1994). Gametophytes (35 mg FW on average in 2 ml culture medium) were incubated in natural seawater supplemented with 10 μM KI. Controls consisted of 20-min incubations, without any additions or in the presence of 10 μM peroxide only, or with the steady-state concentration of 5 μM. The effect of haloperoxidase on iodine uptake was assessed in similar oxidative conditions, yet with addition of 10 $U \cdot m^{-1}$ of A.n. I haloperoxidase or of 6 $U \cdot ml^{-1}$ of haloperoxidase from *L. digitata*. At the end of incubation, gametophytes were washed and centrifuged three times, the amount of accumulated iodine was determined and the uptake of label was compared with the amount removed from the liquid. Experiments were performed in 5–10 replicates.

Table 1. Pharmacological investigation of the relationships between iodine uptake and oxidative metabolism. Tissue disks were preincubated for 1 h in the presence of various compounds known to interfere with the apoplastic concentration of activated oxygen species. Subsequent inhibition (−) or enhancement (+) of the rates of iodine uptake (10 μM KI, 10 min) are reported as percent of the control sporophyte tissue. Ld, *Laminaria digitata*; Ls, *L. saccharina*

Compound or Enzyme	Concn. or Activity	Species	Relative I uptake (%)
H_2O_2	5 μM	Ls	+24
	25 μM	Ls	+46
	50 μM	Ls	+12
	75 μM	Ls	−10
	250 μM	Ls	−33
	500 μM	Ls	−44
Ascorbate	2.5 mM	Ld	−86
	2.5 mM	Ls	−93
Cu-bi-salicylate	500 μM	Ld	−39
	5 mM	Ld	−86
Glutathione	500 μM	Ld	−47
	2.5 mM	Ls	−84
	5 mM	Ld	−94

Results

Iodine accumulation by Laminaria sporophytes. The iodine contents of sporophytes of *L. digitata* collected off Roscoff or cultured in tanks of running-sea water were compared (Fig. 1). The older the sporophytes the lower their average iodine contents, down to a level of 0.4% in 3- to 4-year old plants. In such adult sporophytes, younger, fast-growing tissue tended to have a lower iodine content than older parts. Plantlets (less than 15 cm in length), however, were very rich in iodine, 4.7% DW on average, equivalent to a concentration of 73 mM fresh tissue and an accumulation of 5 orders of magnitude relative to seawater. The total iodine content of *L. saccharina* protoplast preparations ranged from 0.33 to 0.74% in dry weight. Relative to cells in planta and considering that cell walls account for ca. 50% of dry weight in *Laminaria* (Mabeau and Kloareg 1987), protoplasts had lost from 50% to 80% of the intracellular iodine pool.

Iodine uptake by Laminaria sporophytes. The rate of iodine uptake by thallus discs punched out of marginal *L. saccharina* sporophyte tissue depended on the iodide concentration in the external medium. Michaelis-Menten kinetics were observed in the range of 2 μM $^{-1}$mM iodide concentrations (Fig. 2). A 500-fold increase in the iodide concentration in the surrounding medium (2 μM to 1 mM) raised the uptake rate from 2.8 nmol · (g tissue)$^{-1}$ · min^{-1} to 75.6 nmol · (g tissue)$^{-1}$ · min^{-1}, i.e., a 27-fold enhancement. At the highest concentration tested, about 1.5 μmol iodine was taken up per gram of tissue after 20 min. The apparent V_{max} and K_m values observed with tissue discs were 40.8–52.3 nmol · (g tissue)$^{-1}$ · min^{-1} and 26–47 μM iodide, respectively. The *L. digitata* tissue exhibited similar Michaelis-Menten kinetics, with apparent V_{max} and K_m values of 65.4 nmol · (g tissue)$^{-1}$ · min^{-1} and 420 μM iodide, respectively (not shown). Incubation in the presence of 4 μM dichlorophenyl-dimethylurea (DCMU), an inhibitor of the D1 protein of the photosynthetic electron transport chain, slightly enhanced the uptake of iodide. Preincubation for 15–60 min with the proton-gradient uncoupler DNP at concentrations as high as 1 mM did not have a significant inhibitory effect on iodine absorption.

Involvement of activated oxygen species (AOS) in iodine accumulation by Laminaria. In order to investigate the involvement of oxidative metabolism in iodine uptake, *Laminaria* sporophyte tissue discs were incubated with H_2O_2 over concentrations ranging from 5 μM to 500 μM. Enhancement of iodine uptake was highest (46%) upon addition of 25 μM H_2O_2 (Table 1). At H_2O_2 concentrations above ca. 60 μM, however, the uptake of radioactive iodine decreased significantly, down to one-half of control levels at concentrations around 1 mM. Preincubating *Laminaria* tissues for 1 h in the presence of various AOS scavengers such as glutathione, ascorbate or copper bi-salicylate also resulted in a reduction of iodine uptake, by 86–94% from

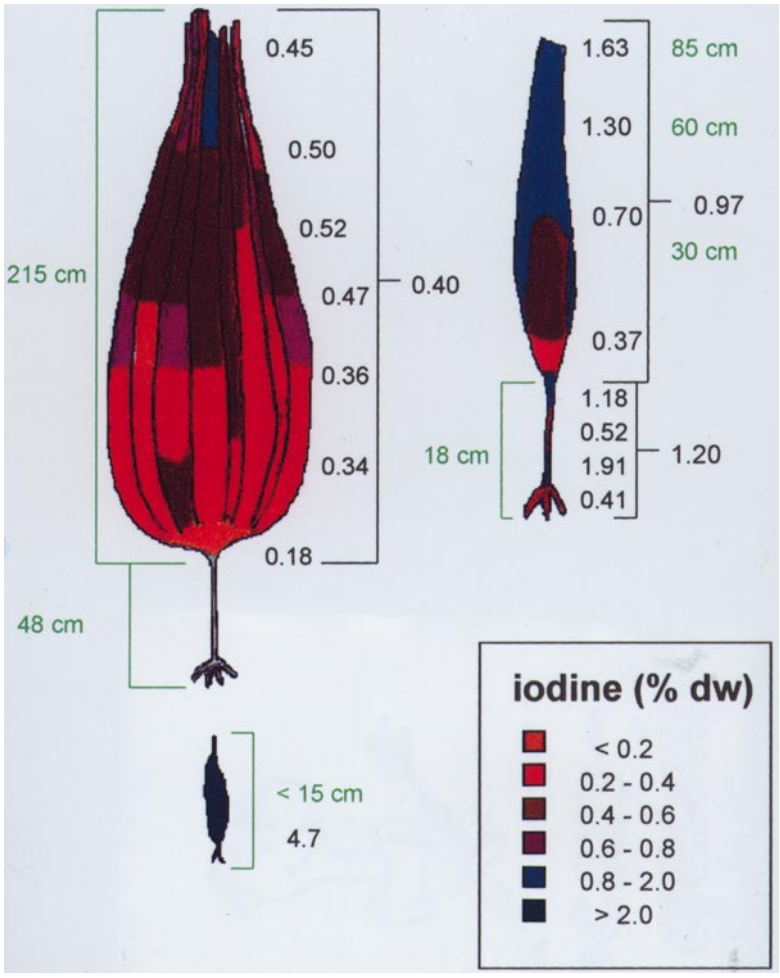

Fig. 1. Accumulation of iodine in sporophytes of *L. digitata*. Plants were collected at Enez Glas in Roscoff in the Spring of 1996. Iodine contents are displayed by different colours (key in the figure). Note that in 1-year-old plants (ca. 1 m long), iodine contents are lower in elongating areas above and below the meristematic zone, as well as in the holdfast. In young plantlets the iodine content, an average of 4.7% of dry weight, represents an accumulation of 150,000-fold relative to natural seawater

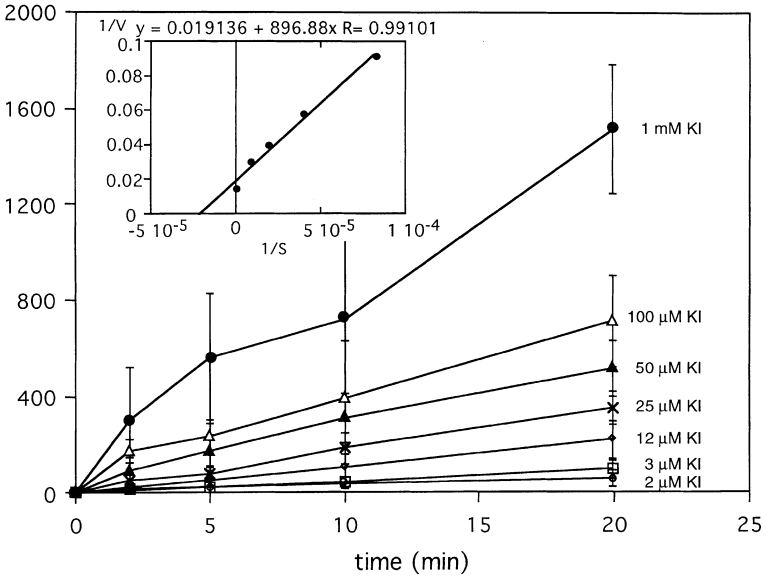

Fig. 2. Kinetics of iodine uptake by *L. saccharina* sporophyte discs in the presence of various concentrations of KI ($n = 5$). The amounts of iodine taken up are given on the ordinate in nanomoles per gram of tissue (FW). Error bars represent the standard deviation from the average value. The Lineweaver-Burk plot used for K_m and V_{max} determination is inserted

control levels (Table 1). The inhibitory effect of ascorbate was investigated over 4 orders of magnitude, between 1 µM and 10 mM. Inhibition of the uptake of iodine became significant at concentrations over 100 µM ascorbate and could be reversed upon removing the scavenger from the medium (not shown).

Laminaria digitata plantlets previously loaded with ^{125}I released iodine at an efflux rate of 1.87 nmol · (g tissue)$^{-1}$ · min^{-1}, corresponding to a loss of 0.15% per hour of the accumulated iodine (based on the average iodine content of such plantlets, see Fig. 1). In contrast, an immediate and marked release of iodine was

Table 2. Release of accumulated iodine upon oxidative stress. Young *L. digitata* plantlets pre-loaded with radioactive label were exposed to various peroxide concentrations. Most of the iodine was usually released within the first 15 min of the stress

H_2O_2 addition	% iodine released after 3 h
Control	0.06 ± 1.41
50 μM	4.16 ± 1.05
200 μM	5.55 ± 2.22
2 mM	7.81 ± 10.29

observable upon incubation in the presence of exogenous H_2O_2, from concentrations higher than 50 μM (Table 2). Incubation with 2 mM H_2O_2 resulted in an initial rate of efflux of 615.2 nmol · (g tissue)$^{-1}$ · min^{-1} and in the release of about 8% of the intracellular iodine reservoir within 10 min.

The uptake of iodine by protoplasts prepared from basal and marginal tissues of *L. saccharina* was compared with that of thallus discs, assuming that 5×10^7 protoplasts correspond to about 1 g of sporophyte tissue (Butler et al. 1989). In the presence of 2 μM iodide, the absorption rate by protoplasts (11 nmol · g^{-1} · min^{-1}) was only one-tenth of that measured in the tissue. However, upon addition of glucose and GOD as an extracellular H_2O_2-generating system, uptake of iodine was sustainably restored in protoplasts (Fig. 3). The rate of uptake sharply increased at a GOD concentration threshold around 0.01 U · ml^{-1} (corresponding to a supply of about 100 μM peroxide), reaching saturation at above 1 U · ml^{-1} (corresponding to steady-state concentrations of about 1 mM peroxide), and leading to the uptake of about 10% of the iodine available from the solution within 10 min. Control experiments using [^{14}C]-sorbitol, a polyalcohol known to be membrane-impermeable, indicated that under these conditions carry-over from the incubation medium was less than 0.6% after 1 h incubation.

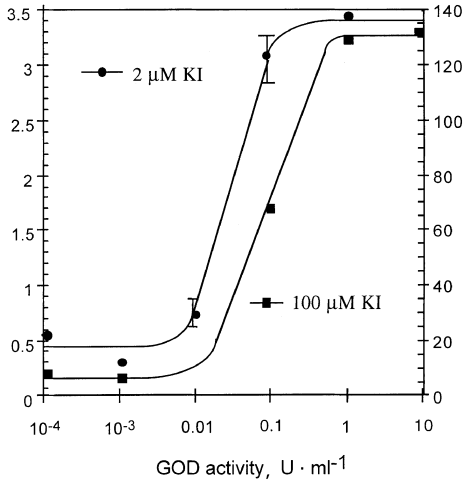

Fig. 3. Influence of the concentration of exogenous GOD on the uptake of iodine by isolated *L. saccharina* protoplasts. Protoplasts were incubated at two different KI concentrations in the presence of 100 mM glucose and of various GOD activities, ranging from 10^{-4}–10 U · ml^{-1}. The amounts of accumulated iodine are given on the ordinates in nanomoles taken up per $1.5 \cdot 10^8$ protoplasts within 20 min, for 2 μM KI (●, left-hand scale) and 100 μM KI (■, right-hand scale)

Involvement of haloperoxidase in iodine uptake. Potential involvement of cell-wall haloperoxidases in iodine uptake (Vilter et al. 1983) was investigated using *L. digitata* gametophytic filaments and vanadate-dependent haloperoxidases purified from *Ascophyllum nodosum* (Fig. 4) and *L. digitata*. Constitutive levels of iodine uptake were much lower than in sporophytes, ca. 0.24 nmol · (g Fw)$^{-1}$ · min^{-1} in the presence of 10 μM KI (Fig. 4, treatment a). When provided with 10 μM exogenous H_2O_2 (Fig. 4, treatment b), gametophytes took up iodide at a rate of ca. 0.87 nmol · (g Fw)$^{-1}$ · min^{-1}. This rate was further increased 2-fold upon addition of haloperoxidase from *A. nodosum* (Fig. 4, treatment c). Even higher uptake rates, up to 2.7 nmol · (g FW)$^{-1}$ · min^{-1}, were obtained in the presence of 10 U · ml^{-1} A.n.I haloperoxidase when a second enzymatic system was added, consisting of 0.025 U · ml^{-1} GOD, 6.4 U · ml^{-1} catalase and 100 mM glucose and generating a constant level of 5 μM peroxide (Fig. 4, treatment e). Comparable results were obtained with the isoenzyme from *L. digitata* (data not shown).

Budget of iodine fluxes in Laminaria. In all uptake experiments with tissue discs, the removal of radioactive label from the medium (as determined by measurements in liquid aliquots) exceeded the activity actually taken up by *Laminaria* sporophytes. The deficit ranged between

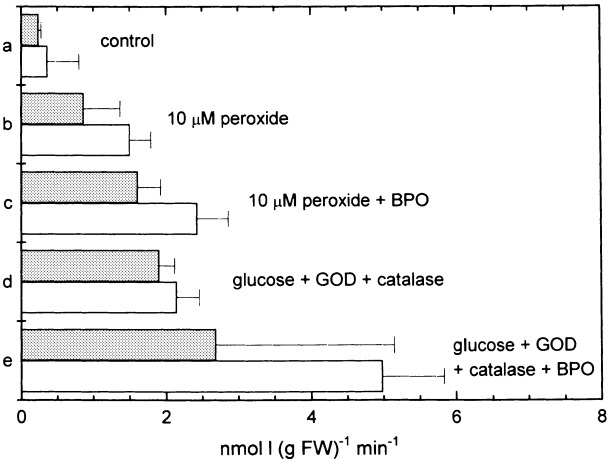

Fig. 4. Effect of haloperoxidase on iodine uptake by *L. digitata* gametophytes. The rates of iodine uptake are given in nmol · (g Fw)$^{-1}$ · min^{-1} (n = 5–10). *Filled bars*, amount of iodine taken up by gametophytes; *open bars*, amount of iodine removed from the medium. The difference for each treatment is presumed to be accounted for by iodovolatilisation. *a*, Controls, consisting of gametophytes incubated for 20 min in the presence of 10 μM KI; *b*, addition of 10 μM exogenous H_2O_2; *c*, addition of 10 μM exogenous H_2O_2 plus 10 U · ml^{-1} exogenous A.n.I haloperoxidase; *d*, addition of 0.025 U · ml^{-1} GOD, 100 mM glucose and 6.4 U · ml^{-1} catalase; *e*, addition of 0.025 U · ml^{-1} GOD, 100 mM glucose, 6.4 U · ml^{-1} catalase and 10 U · ml^{-1} exogenous A.n.I haloperoxidase

10 and 30% of the total iodine removed from the medium. Controls demonstrated that this deficit could not be accounted for by the washing procedure nor by a quenching of (γ-) rays by the setup for detecting radioactivity. A comparable deficit of radioactive iodine was also detected in oxidative efflux experiments with plantlets previously charged with radioactive iodine. Finally, when using *L. digitata* gametophytes in various uptake conditions, the amounts of label removed from the incubation medium were always significantly higher than the label recovered in the biomass (Fig. 4).

Discussion

Iodine uptake in Laminaria is a facilitated-diffusion mechanism. For an iodine concentration of 2 μM KI and upon incubation for 6 h, *L. saccharina* tissue took up iodine at the rate of 1 nmol · g^{-1} · min^{-1}. In a comparable experiment with tissue discs incubated in 1 μM KI the uptake rate was 0.7 nmol · g^{-1} · min^{-1} (Amat 1985). Consistent with the 17-fold enhancement observed by Amat (1985) upon increasing the external iodide concentration from 1 μM to 100 μM, raising the iodide concentration in seawater from 2 to 100 μM and to 1 mM resulted in a 12.8-fold and a 27-fold increase in the iodine uptake rate, respectively. Overall, the variations in the uptake of iodide by *L. saccharina* tissue with the external iodide concentration fit a Michaelis-Menten-like equation (Fig. 1). In agreement with the fact that Laminariales accumulate much more iodine than Fucales (Vilter et al. 1983), the apparent theoretical maximal speeds of iodine entry in kelp sporophyte tissue, ca. 45 nmol · g^{-1} · min^{-1} in *L. saccharina* and 65.4 nmol · g^{-1} · min^{-1} in *L. digitata*, are about 10 times higher than in *F. ceranoides* (V_{max} = 4.2–7.5 nmol · g^{-1} · min^{-1}; Baily and Kelly 1955).

One-hour preincubations with DNP at concentrations as high as 1 mM did not inhibit iodine uptake by *L. saccharina* tissues. Based on the reversible, inhibitory effect of a 20-h preincubation with 500 μM DNP, André (1965) suggested an active transport process for iodide in *L. digitata*. However, as this proton ionophore is likely to disturb the whole ATP-dependent metabolism, secondary effects caused by energy depletion cannot be excluded in such long-term experiments. Indeed, the uptake of iodine was not significantly affected until 6 h after the addition of DNP (André 1965). Similarly, no inhibitory effect was observed on iodine uptake by *L. saccharina* upon incubation in the presence of 10–20 μM carboxyatractiloside, an inhibitor of the mitochondrial ATP translocase which blocks the availability of ATP from respiration (Amat 1985). Altogether, it appears that iodine uptake follows Michaelis-Menten kinetics but that no direct ATP expenditure is required for the accumulation of iodine in *Laminaria*. According to the definition of Lobban et al. (1985), this process may therefore be thought of as a facilitated-diffusion mechanism, by which the rate of transport of iodide into *Laminaria* cells is increased, irrespective of its electrochemical potential gradient.

Apoplastic oxidative power regulates the influx and efflux of iodine. Hydrogen peroxide spontaneously reacts with iodide to form hypoiodous acid (1), virtually undissociated (pK = 10.64) at the pH (8.0) of seawater,

$$I^- + H_2O_2 \Longleftrightarrow HIO + OH^- \quad (1)$$

$$HIO + I^- + H^+ \Longleftrightarrow I_2 + H_2O \quad (2)$$

and itself in equilibrium (2) with molecular iodine in aqueous solutions (Truesdale 1995). If one or the other of the oxidised iodine species can freely cross the plasma membrane, one would expect iodine influx into *Laminaria* cells to be dependent on apoplastic oxidative power. The uptake of iodine by sporophyte tissue was indeed enhanced by low doses of exogenous peroxide. The dose-dependent inhibition of tracer accumulation by scavengers (Table 1) is likely to be due to competition with iodide in accepting electrons from AOS.

Only a very small constitutive net production of H_2O_2, ca. 0.5 nmol · (g Fw)$^{-1}$ · min^{-1}, is required in the apoplast to stoichiometrically account for the uptake of iodine from natural seawater. Assuming the apoplastic volume of *Laminaria* fronds at about 10% of the tissue fresh weight (Mabeau and Kloareg 1987), this basal production amounts to peroxide concentrations in the apoplast of ca. 5 μM. Consistently, *Laminaria* disks provided with exogenous H_2O_2 showed an enhanced uptake for concentrations of peroxide ranging from 5–50 μM. However, attempts to artificially further increase the apoplastic peroxide levels resulted in a marked inhibition of the influx of radioactive iodine (Table 1). As shown in Table 2, this is most likely due to a massive efflux of non-radioactive iodine followed by isotopic dilution of the external tracer. Consistently, methyl viologen, a redox-cycling compound generating massive quantities of AOS in plastids and other cellular compartments, inhibited iodine uptake by almost 30% when applied at the concentration of 20 μM.

In contrast to the blade tissue, protoplasts isolated from *L. saccharina* did not take up iodine at significant rates. In the presence of glucose/GOD, however, iodine influx into protoplasts correlated with the activity of the peroxide-generating system (Fig. 3). As carry-over from the surrounding medium was smaller by 2 orders of magnitude than actual accumulation of iodine, the influx of this element in *Laminaria* protoplasts supplied with H_2O_2 cannot be accounted for by membrane leakiness. However, whilst H_2O_2 concentrations higher than 50 μM were reducing iodine uptake in tissue, concentrations as high as 1 mM still enhanced net iodine uptake in protoplasts (Fig. 3). This probably reflects a significantly lower rate of iodine efflux from the isolated protoplasts, which had lost about half of the intracellular concentration of this element, probably as a result of the massive oxidative stress during enzymatic cell wall degradation (Benet et al. 1997).

As an uncharged and relatively stable molecule, H_2O_2 can easily cross membranes and reach locations remote from the site of its formation. In plant cells, AOS arise in plastids and mitochondria, from photorespiration or incomplete reduction of oxygen. Yet, in *Laminaria*,

neither blocking the electron transport downstream of PSII with dichlorophenyl-dimethylurea nor diverting electrons from $NADP^+$ reduction into the Mehler reaction with methyl viologen markedly increased iodine uptake. Consistently, the influx of iodide is not reduced by darkness (e.g. Shaw 1959; Amat 1985). From these data, intracellular production of H_2O_2 does not appear to be a major source for the oxidation of apoplastic iodide. The AOS also can be generated extracellularly, by plasmalemmal NADPH oxidases (e.g. Baker and Orlandi 1995) or a variety of apoplastic oxidases and peroxidases (e.g., Mader et al. 1980; Dumas et al. 1993; Bolwell et al. 1995). In Laminariales, no plasmalemmal or apoplastic proteins involved in generation of H_2O_2 have been described yet. In particular, no "iodide oxidase" has been isolated so far. Such apoplastic enzymes, however, are likely to be lacking or inactivated in the isolated protoplasts. Restoration of iodine uptake in protoplasts by exogenous peroxide thus indicates that extracellular enzymes are the main providers of AOS for the accumulation of iodine in kelps.

Haloperoxidase catalyses iodine uptake in Laminaria. Murphy and O hEocha (1973b) have suggested that, in addition to a peroxide-generating system, iodide uptake in algae is mediated by an iodide peroxidase, which would catalyse the oxidation of iodide by peroxide. It is now well documented that cell walls of brown algae contain various isoforms of vanadium (V)-containing haloperoxidases (Vilter 1995), with activities as high as ca. 5 $U \cdot ml^{-1}$ in *Laminaria* apoplasm (Jordan et al. 1991). Haloperoxidases are known to catalyse the formation of hypohalides, according to reaction (3),

$$X^- + H_2O_2 \Longleftrightarrow HXO + OH^- \qquad (3)$$

where X^- stands for the halides Br^- or I^- (Vilter 1995). As reaction (3) is identical to equilibrium (1), one might expect that haloperoxidases are involved in iodine accumulation in brown algae (Jordan and Vilter 1991). Consistent with this hypothesis, in the presence of 6–12 $U \cdot ml^{-1}$ haloperoxidase from either *L. digitata* or *A. nodosum* and a constant supply of 5 μM H_2O_2, *L. digitata* gametophytes took up iodine at rates markedly higher than controls and approaching those of sporophytes. As these H_2O_2 levels and peroxidase activities can be thought of as representative of the physiological apoplastic conditions in sporophytes, this finding indicates that haloperoxidase is involved in iodine absorption in kelps, by catalyzing iodide oxidation in the cell wall.

The mechanism and possible functions of iodine accumulation in Laminaria. Based on the above results, Fig. 5 illustrates our current view of the mechanism leading to iodide accumulation in *Laminaria*. Cell wall oxidases or membrane-bound enzymes with an extracellular domain, together with possible intracellular sources, generate a steady flow of H_2O_2 into the apoplast. Cell wall haloperoxidases catalyse the physiological oxidation of iodide into hypoiodous acid and molecular iodine. Oxidised iodine can then cross the plasma membrane. Some of the oxidised iodine, however, evolves into the air as molecular iodine or as volatile halocarbons (VHCs). As pointed out by Kelly and Baily (1951), such a iodovolatilisation is likely to account for the deficit of the radioactive tracer observed in both their and our experiments.

As lipophilic species, hypoiodous acid or molecular iodine diffuse freely through the plasmalemma and enter cells, where they are reduced to iodide or diverted to iodinating organic substrates. In this mechanism the

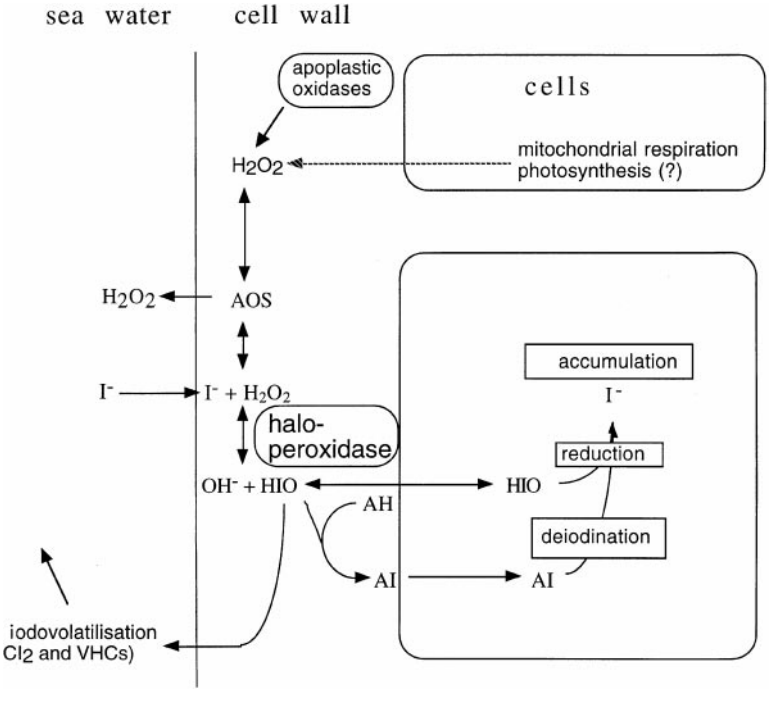

Fig. 5. Diagram showing the possible mechanism of iodine uptake in *Laminaria*. Apoplastic H_2O_2, mainly from extracellular sources is consumed for the oxidation of iodide into hypoiodous acid and molecular iodine, a reaction catalyzed by cell wall haloperoxidase. Oxidised iodide can then freely cross the plasmalemma, resulting in a facilitated diffusion influx of iodine. Haloperoxidase activity may also catalyze the iodination of organic compounds (*AI*) which would act as vectors of iodine into the cells. In steady-state conditions, a net influx of iodine is observed, leading to intracellular accumulation of iodine. Upon oxidative stress, this reservoir is mobilised and a rapid, massive efflux of iodine occurs. In both cases, oxidation of iodide results in the evolution of molecular iodine and of volatile halogenated compounds *VHCs* ("iodovolatilisation")

form of iodine transported across the plasma membrane is oxidised iodide whereas the intracellular stores of iodine mainly consist of reduced or organic iodine. Such a mechanism whereby iodine does not penetrate the cells against an electrochemical potential gradient is consistent with the facilitated diffusion kinetics reported above. This model extends the diagram put forward by Shaw (1959, 1960, 1962), with the addition of two refinements, i.e., the demonstration that oxidation of iodide takes place in the apoplast and that it is catalysed by haloperoxidase. At the thallus level, the distribution of accumulated iodine in *Laminaria* (Fig. 1) can be thought of as the result of two major fluxes, transport into the cells, mediated by both oxidases (still to be identified) and haloperoxidase, as well as long-distance transport (Amat and Srivastava 1985).

One puzzling question remains, the biological significance of the accumulation of iodine by kelps, up to levels as high as 73 mM in *L. digitata* plantlets. It has been proposed (e.g. Wever et al. 1991) that it serves the production of iodinated, antimicrobial molecules. A first defence line would then be the production of H_2O_2 in the apoplast. Such an oxidative burst would in turn trigger a rapid, massive mobilisation of iodine from the intracellular reservoir (this study), leading to the haloperoxidase-mediated formation of VHCs (Wever et al. 1991; Collén et al. 1994; and Fig. 4, this study) and participating in the protection against oxidative stress (Pedersén et al. 1996). The haloperoxidases of brown algae therefore appear to be key enzymes in iodine metabolism, both for its uptake and for its utilization in the production of VHCs.

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