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Mass spectrometry – based imaging techniques for iodine-127 and iodine-129 detection and localization in the brown alga *Laminaria digitata*

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ABSTRACT

^{129}I is one of the main radioisotopes of iodine derived from the nuclear fuel cycle that can be found sustainably in the environment due to its long half-life. In coastal marine environment, brown macroalgae, such laminariales (or kelps), are known to naturally feature highest rates of iodine accumulation, and to be an important source of biogenic volatile iodinated compounds released to the atmosphere. These seaweeds are therefore likely to be significantly marked by but also potential vectors of radioactive iodine. In order to better understand the chemical and isotopic speciation of iodine in brown algal tissues, we combined mass spectrometry-based imaging approaches in natural samples of *Laminaria digitata* young sporophytes, collected at two different locations along the south coast of the English Channel (Roscoff and Goury). Laser desorption ionization (LDI) and desorption electrospray-ionization techniques (DESI), coupled with mass spectrometry, confirmed the predominance of inorganic I^- species on the surface of fresh algae, and a peripheral iodine localization when applied on micro-sections. Moreover, radioactive isotope ^{129}I was not detected on plantlet surface or in stipe sections of algal samples collected near Roscoff but was detected in *L. digitata* samples collected at Goury, near La Hague, where controlled liquid radioactive discharges from the ORANO La Hague reprocessing plant occur. At the subcellular scale, cryo-fixed micro-sections of algal blade samples from both sites were further analyzed by secondary ion mass spectrometry (nano-SIMS), leading to similar results. Even if the signal detected for ^{129}I was much weaker than for ^{127}I in samples from Goury, the chemical imaging revealed some differences in extracellular distribution between radioactive and stable iodine isotopes. Altogether LDI and nano-SIMS are complementary and powerful techniques for the detection and localization of iodine isotopes in algal samples, and for a better understanding of radioactive and stable iodine uptake mechanisms in the marine environment.

1. Introduction

In coastal marine environment, the biogeochemical cycling of iodine is controlled by intense exchanges in the marine boundary layers, from oceans to the atmosphere (McFiggans et al., 2010). From the viewpoint of global circulation, the introduction of iodine radioisotopes by human activities has potential impact on the environment and marine life (Muratmatsu et al., 2004; Schiermeier 2011). The main iodine radioisotopes generated by human activities 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 mainly generated in power plants to produce electricity and is also routinely used for medical treatments in cancer radiotherapy. ^{129}I is produced in power plants and, because of its long half-life, is the major remaining iodine radioisotope of nuclear fuel recycling, as well as fallout from atmospheric nuclear weapon testing and major nuclear power plant accidents.

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Brown macroalgae, such as Laminariales (also called kelps), are known to concentrate iodine up to 10^5 times in young plantlets with regards to seawater content with no rival known among living organisms (Küpper et al., 1998). Furthermore, in response to stress, for example during emersion at low tide, brown seaweeds are an important source of biogenic volatile iodinated compounds and molecular iodine released to the atmosphere (Ball et al., 2010; McFiggans et al., 2010). They are suspected to be one of major contributors of iodine cycling in coastal areas because of their ecological importance along the coasts (La Barre et al., 2010).

Controlled amounts of liquid ^{129}I are routinely released in the English Channel by the ORANO nuclear fuel reprocessing plant of La Hague (Normandy, France). Annual amounts of I-129 discharges are publicly available (https://www.orano.group/en/group/reference-publications), they started in the early 90′ and reached between 1 and 2 TBq.yr^{-1} as of the mid-90'. Because of its very slow decay this radionuclide is sustainably present and monitored in the marine environment (Fiévet et al., 2020). In the English Channel, brown seaweeds are therefore significantly marked by but also potential vectors of radioactive iodine since some ^{129}I re-emitted to the atmosphere may return to the terrestrial environment and human populations. However, data on iodine speciation and accumulation in the marine algal compartment still remain poorly documented. In the kelp *Laminaria digitata*, chemical imaging techniques provided evidence that the distribution of iodine in algal tissue is highly heterogeneous (Verhaeghe et al., 2008). Iodine is mainly stored in the peripheral tissue and appears to be localized in the extracellular space of the apoplasm. These observations partly challenged the uptake mechanism proposed by Shaw (1959) and refined by Küpper et al. (1998), which involved intracellular storage. Here, we tested different analytical techniques based on time-of-flight mass spectrometry (TOF-MS) for quick detection of both iodine species and isotopes in freshly-harvested *L. digitata* samples. In addition, we attempted to apply MS-based chemical imaging techniques to discriminate iodine isotopes and compare the distribution of ^{127}I and ^{129}I in *L. digitata* samples exposed or not to radioactive liquid discharges from the ORANO plant.

2. Material and methods

2.1. Algal materials

Laminaria digitata were harvested at low tide on the shore at Roscoff (Brittany, France) and at Goury (Cape La Hague, Normandy, France), in February 2013 (see Sup. Fig. 1 for geographical location). *L. digitata* young plants (~5 cm in length) were collected *in situ*, transported to the laboratory and maintained in culture rooms up to 7 days, at 13 °C in running filtered sterile local seawater (FSW) with an illumination of 60 μE m^{-2}. s^{-1} and a photoperiod of 12:12 light/dark. These are typical cultures conditions for kelps, which allow to keep young sporophytes alive and healthy, without any stress. The algae were used for chemical fixation or shipping, alive in seawater, for DESI- and LDI-MS, or for cryofixation and the subsequent nano-SIMS analyses.

2.2. Time-of-flight (TOF) mass spectrometry analysis

Two techniques based on time-of-flight mass spectrometry were used to analyze iodine chemical and isotopic speciation at the "Service d'Etude du Comportement des Radionucléides" at CEA, Saclay. The desorption-electrospray-ionization technique coupled with mass spectrometry (DESI-MS) was applied to a fresh young plantlet fixed with double-side tape on a glass slide, using MeOH/H_2O (v/v, 50/50) as desolvatation solvent. For laser desorption ionization, coupled with mass spectrometry (LDI-MS), whole young plantlets or transversal or longitudinal micro-sections of algal stipes were fixed on conductive glass thanks to conductive double face tape and directly analyzed under vacuum.

DESI-MS experiments were carried out using an LCT XE Premier (Waters, Manchester, UK) equipped with the DESI ion source Omnispray from Prosolia (Indianapolis, IN). The infusion syringe pump delivered the solvent MeOH/water (50/50). LDI-MS experiments were carried out using an AutoFlex Speed (Bruker Daltonics) employing 1-kHz Nd:YAG laser. Both DESI and LDI spectra were recorded in negative mode.

2.3. Secondary ion mass spectrometry (SIMS) nanoprobe analyses

Algal samples were prepared for nano-SIMS analyses, using cryofixation or chemical fixation procedures. The cryofixation procedure, first developed for animal tissue preparation by Guerquin-Kern et al. (2004), was applied on algal samples as previously described in Verhaeghe et al. (2008). For chemical fixation, cross sections (2 mm thick) from the blade and the stipe were fixed in 1.5 mL FSW containing 3% (v/v) glutaraldehyde for 3 h at 4 °C and then transferred in 1.5 mL FSW containing 1% paraformaldehyde at 4 °C overnight. Fixation was followed by three 5 min rinses in seawater and by a series of 2 × 30 min rinses in seawater/ethanol solution. In this later solution, the percentage of ethanol was increased by 25% in each successive step. The samples were then dehydrated in ethanol, infiltrated by Spurr's resin for 4 days and, finally, polymerized.

Nano-SIMS analyses were performed using the NanoSIMS-50TM ion microprobe in scanning mode (CAMECA, Genevilliers, France) at the Ion Microscopy Platform of Institut Curie (Orsay, France) as previously described (Verhaeghe et al., 2008). The masses of $^{127}I^-$ and $^{129}I^-$ are so close which prevent their simultaneous detection. Therefore, in the present study, two sequential imaging runs were conducted, the first one with $^{13}C^{14}N^-$, $^{32}S^-$, $^{127}I^-$ and the second one with $^{13}C^{14}N^-$, $^{32}S^-$, $^{129}I^-$ by keeping the same magnetic field and by moving the same detector to the corresponding radius for $^{127}I^-$ and $^{129}I^-$, respectively.

3. Results and discussion

3.1. Analyses by DESI and LDI coupled with mass spectrometry, confirmed predominance of inorganic I^- species on the surface of fresh *L. digitata*

A feasibility study evaluated the DESI-MS technique to study *in vivo* iodine speciation in *L. digitata*. The spectrum (Fig. 1A) corresponding to the entire plantlet surface, i.e. both blade and stipe surfaces, mainly revealed inorganic iodide species. However, even though the analytical DESI-MS set up was straight forward and allowed to acquire spectral data from living young algae, it showed some important limitations in relation to the nature of the marine samples. The analysis conditions, at room temperature, induced stress by exposure the plantlet to the air, leading to saturating levels of iodine. Another technique coupled with mass spectrometry, LDI-MS, was then applied on a frozen stipe section of *L. digitata*, confirming a similar pattern with the predominance of iodide (Fig. 1A), as already found in this species using X-ray absorption spectroscopy (Küpper et al., 2008).

3.2. DESI- and LDI-MS revealed the presence of $^{129}I^-$ only in algal samples from Goury

Interestingly, these two techniques (DESI- and LDI-MS) also detected signals corresponding to the radioactive isotope ^{129}I, but at a lower level compared to ^{127}I and only in samples of algae harvested at Goury, near the Cape la Hague (Fig. 1B). Though ^{129}I from natural origin as well as from the fallout of past atmospheric nuclear weapon tests is present, it remained undetected in spectra obtained from whole surface plantlet and from stipe micro-sections of *L. digitata* harvested at Roscoff, located in South-West of English Channel, at 200 km from la Hague. This is consistent with the general water mass movement in the English Channel which results in an eastward drift and keeps Roscoff poorly influenced by radioactive discharges from the ORANO plant in the Cape La Hague (Bailly du Bois and Dumas, 2005).

LDI-MS was then used to analyze the iodine chemical and isotopic speciation at the surface of a plantlet collected at Goury. The peak

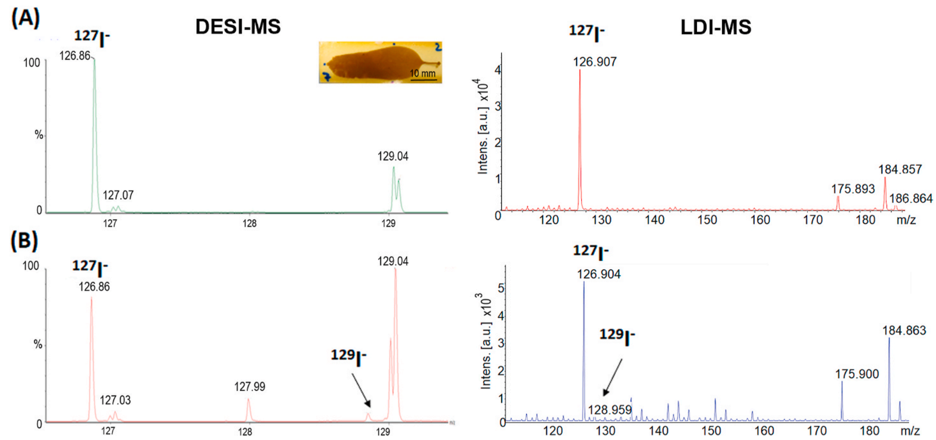

Fig. 1. Iodine isotopic speciation mass spectra obtained by DESI-MS (left panel) analysis for mass range m/z = 127–129 of the whole plantlet surfaces and by LDI-MS (right panel) analysis for mass range m/z = 110–190 analysis of a stipe microsection of *L. digitata*, harvested at (A) Roscoff and (B) Goury.

of $^{129}I^-$ was undetectable in LDI-MS spectrum from the blade surface (Fig. 2A), at the contrary of the stipe surface analysis where both isotopes were visible in the spectrum (Fig. 2B). As a small peak of $^{129}I^-$ was visible on the DESI-MS spectrum obtained from the whole plantlet surface harvested at Goury (Fig. 1B), this result suggested that the LDI-MS technique is near the limit of detection of $^{129}I^-$ species for blade sample analysis, and is only efficient for detecting this radioisotope in algal tissue samples showing the strongest iodine content, as already shown in stipe peripheral tissues of *L. digitata* (Verhaeghe et al., 2008).

3.3. Peripheral iodine localization in micro-section LDI-MS imaging

Applied on cross-sections, the LDI-MS technique allowed accessing the chemical and isotopic speciation as well as location information of specific chemical element based on mass spectrum data. A preliminary result was obtained on a 25 μm frozen micro-section of *L. digitata* stipes, collected at Roscoff (Sup. Fig. 2). The average spectrum obtained over the entire analyzed zone under vacuum revealed that only inorganic iodine forms were detected, and mainly I^- corresponding to m/z 126.9 ion ($^{127}I^-$) as already shown. Based on this m/z, mass spectral imaging showed a main iodide localization at the periphery of the section. This peripheric distribution of iodine is in agreement with previous proton microprobe imaging using particle-induced X-ray emission (PIXE) (Verhaeghe et al., 2008). With spatial resolutions down to 2 μm, this latter technique offered a ten-fold better spatial resolution than LDI-MS imaging. However, LDI-MS analysis gave access to the isotopic speciation of iodine with the detection of ^{129}I, when applied on stipe sections of *L. digitata* collected at Goury (Fig. 3). On this longitudinal section, both $^{127}I^-$ and $^{129}I^-$ ions are located in the external tissues of the alga, suggesting a co-localization of these two isotopes at the 20 μm resolution of LDI-MS imaging.

3.4. Nano-SIMS imaging suggested a different extracellular distribution between radioactive and stable iodine isotopes

In order to study iodine isotope distribution at the subcellular scale, samples from both sites were also analyzed on the nano-SIMS platform of the Institut Curie at Orsay (France). The preservation of speciation and distribution of iodine species, especially labile ones such as iodide, required the use of cryofixation methods, as already discussed in Verhaeghe et al. (2008). In cryo-fixed preparations of *L. digitata* blades from both sites, the chemical imaging of ^{127}I confirmed an apoplastic location, i.e. extracellular, and featured a huge and maximum signal at the level of the mucilage (Fig. 4), as previously shown (Verhaeghe et al., 2008). Whereas the signal for ^{129}I was undetectable in *L. digitata* blades collected at Roscoff (Fig. 4A), in agreement with DESI- and LDI-MS analyses, it was weak, but quantifiable, mainly in apoplasm of *L. digitata* samples collected at Goury (Fig. 4B). Interestingly, when comparing the apoplastic distribution of iodine isotopes in this cryofixed microsection, ^{129}I signal presented a much less pronounced gradient

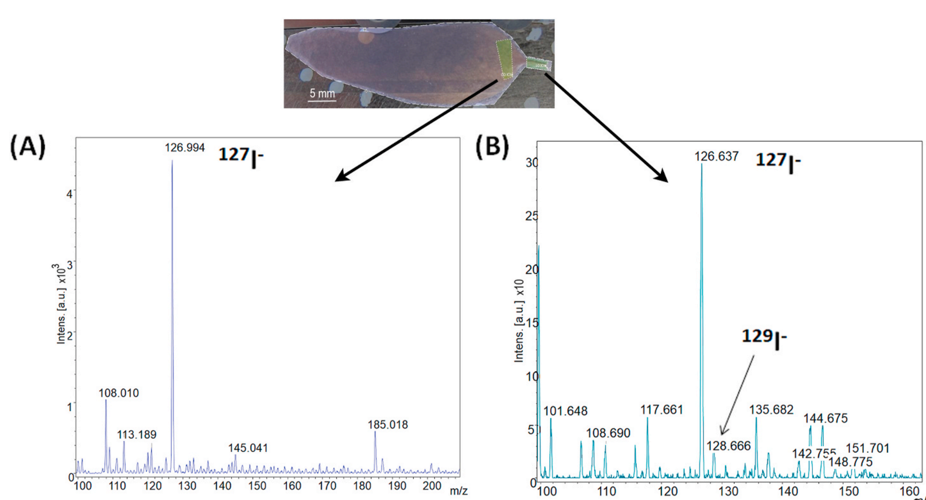

Fig. 2. Iodine isotopic speciation mass spectra obtained by LDI-MS analysis of surfaces of a *L. digitata* plantlet harvested at Goury. (A) Blade zone for mass range m/z = 100–220, and (B) Stipe zone for mass range m/z = 100–165.

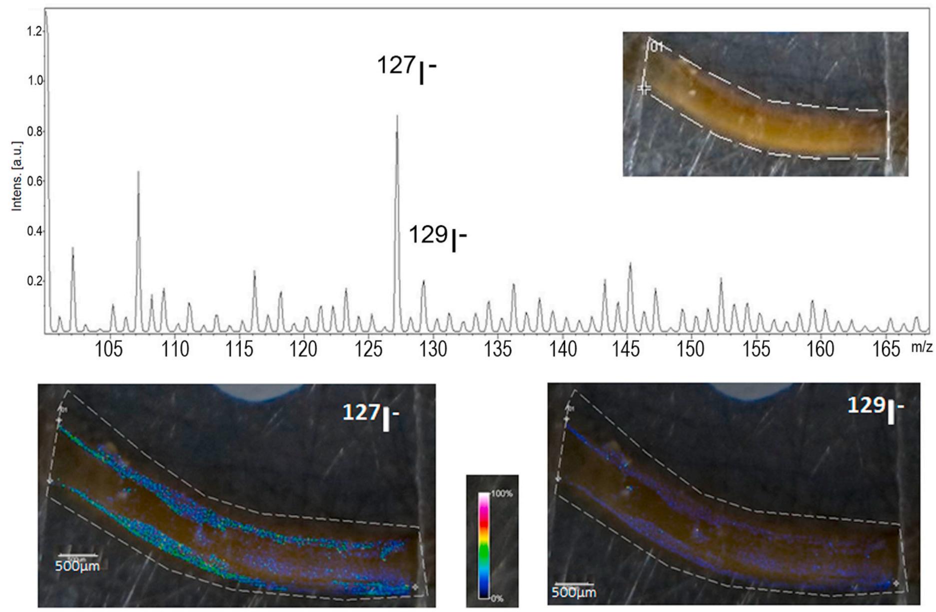

Fig. 3. Iodine isotopic speciation mass spectra acquired by LDI-MS analysis for mass range m/z = 100–170 and selected ion image obtained for $^{127}I^-$ and $^{129}I^-$, from longitudinal stipe section of *L. digitata* harvested at Goury.

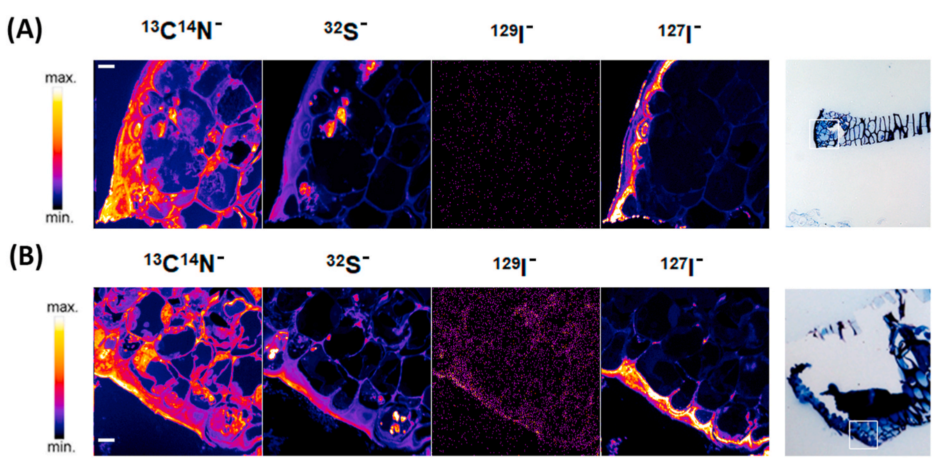

Fig. 4. Nano-SIMS imaging showing iodine subcellular localization and isotopic distribution of ^{129}I and ^{127}I in peripheral zones of cryofixed blade sections of young *L. digitata* collected at (A) Roscoff and (B) Goury. Nano-SIMS images are colored in a relative linear scale of value levels for each element. At the right the corresponding histological blade sections are represented with white squares indicating the two analyzed zones. Nano-SIMS image size: 50 µm × 50 µm. Data acquisition times were 10 min for $^{127}I^-$, $^{13}C^{14}N^-$, $^{32}S^-$ in a first run and 150 min for $^{129}I^-$, $^{13}C^{14}N^-$, $^{32}S^-$ in a second run. Scale bar: 5 µm.

than ^{127}I signal, in the mucilage (Fig. 4B). In addition, we have conducted nano-SIMS analyses on chemically-fixed stipe microsections. In these imaging, the iodine signal resulted from the non-labile forms of iodine, which were also mainly visible in the apoplast for both iodine isotopes (Sup. Fig. 3). The ^{129}I signal was detected weakly, and uniformly in Roscoff's and in Goury's chemically-fixed microsections of stipes, without a significant stronger concentration of this isotope in the external mucilage (Sup. Fig. 3). As this part is thought to concentrate the majority of labile iodine species (Verhaeghe et al., 2008), chemical fixation treatments could have significantly washed off iodide radioactive species, which then seem to be present in significant lower amount compared to ^{127}I organic species.

For ^{129}I, as the signal intensity was very low, the acquisition time was increased by a 15-fold factor compared to the one for ^{127}I. Nevertheless, nano-SIMS was efficient for detecting and localizing labile ^{129}I species in a cryofixed blade sample or ^{129}I strongly bounded organic species forms in a chemically-fixed stipe microsection of *L. digitata*. While the distribution of ^{127}I labile and organic species was similar between cryofixed and chemically-fixed samples, it was not the case for ^{129}I, whose distribution is further not correlated with that of ^{127}I. These isotopic discrepancies suggest different mechanisms of chemical retention for iodine species in the surface layers of *L. digitata*. It should be outlined that differences in the isotopic ratio of I^- and IO_3^- in seawater from the English Channel had been previously reported by Hou et al. (2007). Interestingly, our observations on iodine isotopic distribution in *Laminaria* compartments are potentially promising since preliminary measurements of both stable ^{127}I and radioactive ^{129}I performed by IRSN in seawater and whole brown seaweeds from the Cap de La Hague area also showed discrepancies in the isotopic ratio of the different inorganic iodine forms (I^-, IO_3^-) (B. Fiévet and C. Voiseux, pers. comm.).

4. Conclusion

We have shown that it was possible to detect, and potentially quantify, iodine-129 isotope in fresh algae harvested in the environment, near La Hague, in the English Channel, either by DESI-MS and LDI-MS (direct and rapid methods, but less sensitive with a lower spatial resolution), or by nano-SIMS (data on subcellular distribution). Our results showed that ^{127}I and ^{129}I isotopes featured peripheral tissue and apoplastic subcellular localizations in *L. digitata*. In addition, nano-SIMS imaging suggested a different extracellular distribution of radioactive isotopes. Beyond this observed isotopic fractionation in tissues of *L. digitata*, these results raise questions about the preferential form absorbed by the algae (iodide or other oxidized forms), and the part of the perennially fixed *versus* labile forms of iodine in algal tissues. It also highlights the potential of noninvasive *in vivo* chemical analysis, such as LDI-MS and nano-SIMS, to further explore the concentration mechanisms of iodine by kelps. It will be essential to continue these analyzes to better understand the differences between the observed isotopic ratios and to further study the kinetics of incorporation of ^{129}I radioactive isotope into algae tissues in the natural environment, near the Cape of La Hague.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvrad.2021.106552.

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