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First Time Identification of Selenoneine in Seabirds and Its Potential Role in Mercury Detoxification

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extremely high concentrations of Se are found. Selenium speciation in biota has aroused great interest in recent years; however, there is a lack of information about the chemical form of Se in (sea)birds. The majority of publications focus on the growth performance and antioxidant status in broilers in relation to Se dietary supplementation. The present work combines elemental and molecular mass spectrometry for the characterization of Se species in wild (sea)birds. A set of eight giant petrels (*Macronectes* sp.) with a broad age range from the Southern Ocean were studied. Selenoneine, a Seanalogue of ergothioneine, was identified for the first time in wild avian species. This novel Se-compound, previously reported in fish, constitutes the major Se species in the water-soluble fraction of all of the internal tissues and



blood samples analyzed. The levels of selenoneine found in giant petrels are the highest reported in animal tissues until now, supporting the trophic transfer in the marine food web. The characterization of selenoneine in the brain, representing between 78 and 88% of the total Se, suggests a crucial role in the nervous system. The dramatic decrease of selenoneine (from 68 to 3%) with an increase of Hg concentrations in the liver strongly supports the hypothesis of its key role in Hg detoxification.

KEYWORDS: selenoneine, procellariiform, petrels, mercury, selenium, brain, liver

INTRODUCTION

Selenium (Se) is an essential element for living organisms, including humans,^{1,2} due to its central function in selenoenzymes and selenoproteins.³ More than 25 selenoproteins have been identified in life forms from archaea to mammals.⁴ This element plays key metabolic roles;^{3,5} it has been recognized as an antioxidant,^{6,7} and its presence is related to the reduction of certain types of cancer and other diseases.^{4,8–10} Notwithstanding its beneficial effect on living organisms, Se exhibits an ambivalent behavior due to a narrow concentration range that separates the conditions of deficiency in dietary intake and toxicity.¹¹ Not only its concentration but also the chemical form (speciation) determines its potential toxic/beneficial effects.^{12,13}

Birds are principally exposed to Se through diet,¹⁴ and its bioaccumulation is influenced by both intrinsic (e.g., gender, age, long lifespan)^{15,16} and extrinsic (e.g., feeding habitat, diet, migration) factors.^{17,18} Seabirds usually exhibit higher Se levels than terrestrial birds,^{19,20} and they have been used as bioindicators of metallic^{21–23} and organic pollutants.²⁴ Seabirds belonging to the Procellariiformes order include albatrosses and petrels, which are at the top of the list of living organisms exhibiting the maximum Se concentrations.²⁵

These high concentrations in seabird tissues (for example, values over 1100 μ g.g⁻¹ dry weight (dw) in the liver of giant petrels *Macronectes* spp.)²⁶ are likely due to their high position in the marine food web and their long lifespan.^{16,18}

Selenium speciation in biota has aroused great interest in recent years;²⁷ however, there is a lack of information about the chemical form of Se in (sea)birds. The literature concerning Se speciation in birds^{26,28–33} has been almost exclusively focused on experimental birds and broilers.^{28–30,33} Most of these studies compared the growth performance, meat yield, and antioxidant status in broilers depending on the Se dietary sources.^{28,29,34–36} In general terms, a supplementation with organic forms, usually selenomethionine, is more efficiently incorporated than that with selenite, also leading to improved growth and antioxidant status.^{29,33,35} Selenium

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speciation studies in experimental birds (by high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS)) reveal a dissimilar metabolization of inorganic and organic Se sources, as well as an association of Se with high- and medium-molecularweight species.^{28,30,33} Only a few investigations considered Se speciation in wild birds, these being limited to the identification of its association with Hg.^{26,31,32} In this respect, Hg bound to tetraselenolate (Hg(SeR)₄) likely acts as a precursor of tiemannite (HgSe).^{26,31} This latter compound is considered the final product of methylmercury (MeHg) detoxification.³⁷ However, the metabolic pathways involved in the formation of tiemmanite remain unknown.

In the present work, the combination of elemental and molecular mass spectrometry was exploited in the characterization of soluble Se species for the first time in wild seabirds. Specifically, giant petrels (*Macronectes* sp.) with a broad age range were investigated. Different internal tissues, including the liver, kidneys, muscle, brain, testicles, and also the blood of this seabird species, were studied. The potential role of Se species in the Hg metabolism of giant petrels was addressed.

MATERIALS AND METHODS

Sample Collection. Eight dead giant petrels were opportunistically collected from the Kerguelen archipelago (Southern Indian Ocean) and Adélie Land (Antarctic continent). All details regarding the sampling data set are presented in Table S1. The age of these birds could not be precisely determined, but one of the seabirds collected in Adélie Land was a fully-fledged chick, the P-P1 (viz. less than 1 year old). In addition to this, samples of three other *Macronectes giganteus* (see details in Renedo et al.)³⁸ were also considered in the present study (PGA01, PGA02, and PGA03).

The samples from Adélie Land were in good shape for the adult birds (P-10 and P-11) that died after a collision against an electric pole, but the chick (P-P1) was highly emaciated, suggesting that it died from starvation. As for the other birds, the cause of death is unknown; in general, they were in good shape, except for P1 and P3 (emaciated). All of them were collected immediately after their death.

After dissection, all of the samples were stored at -80 °C. The internal tissues analyzed in this study (liver, n = 7; kidneys, n = 5; brain, n = 3; pectoral muscles, n = 6; and testicles, n = 1) were sampled, weighed, and stored individually in plastic bags. Blood samples were obtained by collecting clotted blood from the heart atria and stored in Eppendorf microtubes.

Reagents and Standards. All solutions were prepared using Milli-Q (ultrapure) water (18.2 M Ω cm, Millipore Bedford, MA), and all analytical reagent grade chemicals were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). Trace metal grade acids (HNO₃ and HCl) were purchased from Fisher Scientific (Illkirch, France), and Se standard was purchased from SCP SCIENCE (France). Working standard solutions were prepared daily by appropriate dilution of stock standard solutions in 2% HNO₃ and stored at 4 °C until use.

Instrumentation. Liquid chromatographic separations were carried out with an Agilent 1100 liquid chromatograph (Agilent, Wilmington, DE) equipped with an autosampler and a binary HPLC pump. An Agilent inductively coupled plasma mass spectrometer (ICP-MS) 7500ce (Yokogawa Analytical

Systems, Tokyo, Japan) was used for Se and Hg detection after liquid chromatography separation and for Se quantification after acid digestion of the biological samples. Two sizeexclusion chromatography (SEC) columns were employed: Superdex 200 and Superdex peptide HR 10/30 (10 \times 300 mm², 13 μ m, GE Healthcare, Uppsala, Sweden). The hydrophilic interaction chromatography (HILIC) separation system with a TSKgel Amide-80 column (250 \times 1 mm², 5 μ m, Tosoh Biosciences, Stuttgart, Germany) was coupled to ICP-MS or an electrospray hybrid linear trap quadrupole Orbitrap Velos mass spectrometer (ESI LTQ Orbitrap MS) from Thermo Fisher Scientific (Bremen, Germany) using a heated electrospray ionization source (H ESI II) (Thermo Fisher Scientific).^{39,40} An ultrasonic probe-Vibra-Cell (USP)75115 (Bioblock Scientific, Illkirch) instrument with a 3 mm diameter and a nominal power of 500 W-was used for the extraction of water-soluble fractions. A centrifugation system using a MiniSpin plus model centrifuge (Eppendorf, Hamburg, Germany) at 14 100g was used. The total Hg was determined using cold vapor atomic fluorescence spectrometry (PSA Millennium Merlin 10.025).

Total Se and Hg Determination. Freeze-dried and homogenized tissue samples (0.05-0.2 g) corresponding to the bulk and the water-soluble fraction (details in the section below) were digested (in triplicate) with 1.5 mL of HNO₃ (ca. 63%, analytical grade) after overnight predigestion at room temperature in an ultraWAVE, Milestone Srl system (Table S2). For Se analyses, the resulting solutions were appropriately diluted with Milli-Q water and analyzed by ICP-MS according to the conditions given in Table S3. The measurement of total Hg (THg) in the digests was performed by the atomic fluorescence method with previous cold vapor generation of Hg(0) by reduction with 3% SnCl₂ (Scharlab, Barcelona, Spain) in 10% HCl (J.T. Baker, Fisher Scientific, Illkirch, France) in Milli-Q water. The samples were then diluted in 5% HCl for analyses, and the quantification was performed by standard calibration. Measurements were validated using certified reference materials (DOLT-5, CE-101, BCR-464, and TORT-2). The information relative to the quality assurance of the analyses of Hg and Se is found in Table S4. Concentrations of both elements are further reported in $\mu g g^{-1}$ dw.

Se Speciation. The water-soluble fraction was extracted from fresh samples (approximately 0.1 g) by ultrasonication (30 s at 21% at 100 W) in 3 mL of 100 mM ammonium acetate (pH 7.4), followed by centrifugation, as described previously reported.⁴¹ The resulting water-soluble fraction was analyzed by size-exclusion chromatography (SEC) coupled to ICP-MS under conditions presented in Table S3.

In parallel, to identify low-molecular-weight Se species, an aliquot of the water-soluble fraction was diluted with acetonitrile (1:2 v/v). The mixture was centrifuged at 14 000g for 10 min, and the obtained supernatant was directly analyzed by hydrophilic interaction liquid chromatography (HILIC) coupled to ICP-MS and electrospray ionization (ESI)-MS.^{39,40} Derivatized samples were also prepared, with iodoacetamide and tris(2-carboxyethyl) phosphine (TCEP) as detailed by Klein et al.⁴⁰

ESI-MS Conditions: μ **HILIC-ESI-MS.** The gradient used for the chromatographic separations is described in Table S3. The ESI-MS was operated in the positive ion mode under the following optimum settings: an ion spray voltage of 2.80 kV; a capillary temperature of 300 °C; a capillary temperature of 285

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tissue	individual	Se $\mu g g^{-1}$	Hg μ g g ⁻¹	molar ratio Se/Hg	water-soluble Se (% of total Se)	SEN (% of water- soluble Se)	SEN (% of total tissue Se)	SEN μg Se g^{-1}
liver	P-P1 ^c	19 + 3	1.11 + 0.04	44	70	96	68	13 + 2
	P-10	15 ± 3	109 ± 19	2.7	45	99	45	10 ± 2 52 ± 7
	D.11	113 ± 17 00 ± 21	107 ± 17 00 ± 22	2.7	41	00	40	32 ± 7
	DC A01	$\frac{99 \pm 21}{112 \pm 15}$	214 ± 24^{b}	1.3	15	99	15	$+0 \pm 9$
	D 1	113 ± 13 142 ± 2	214 ± 34	1.5	13	90 86	13	17 ± 2 27 ± 1
	PC 102	142 ± 3	324 ± 08	1.1	19	80	17	27 ± 1
	PGA05	$222 \pm 2/$	403 ± 7	1.4	18	90	17	30 ± 3
	P-3	420 ± 3	928 ± 73	1.2	3	97	3	12.8 ± 0.1
kidneys	P-PT	41 ± 5	0.55 ± 0.15	185	76	95	72	30 ± 4
	P-10	109 ± 10	25 ± 3	11	81	96	78	85 ± 8
	P-11	116 ± 21	32 ± 1	18	78	97	76	88 ± 13
	PGA02	80 ± 7	39.9 ± 0.4^{b}	5.1	43	76	32	26 ± 2
	PGA03	131 ± 10	50.8 ± 0.7^{b}	6.5	53	91	49	64 ± 5
muscle	P-P1 ^c	4 ± 3	0.10 ± 0.03	92	57	85	48	2 ± 1
	P-10	12 ± 2	1.0 ± 0.2	30	74	93	68	8 ± 2
	P-11	13 ± 3	1.4 ± 0.2	24	70	93	65	9 ± 2
	PGA01	25 ± 4	4.1 ± 0.1^{b}	15	70	99	69	25 ± 3
	P-1	30 ± 1	37 ± 6	2.1	25	81	20	6.0 ± 0.1
	PGA03	31 ± 3	29.2 ± 0.3^{b}	2.8	58	98	56	18 ± 2
brain	P-P1 ^c	6 ± 5	0.19 ± 0.11	77	84	93	78	5 ± 4
	P-10	40 ± 5	1.40 ± 0.66	74	86	98	85	35 ± 4
	P-11	40 ± 2	3.1 ± 0.1	33	89	99	88	35 ± 2
blood	P-P1 ^c	3 ± 1	0.07 ± 0.02	90	80	76	61	1.2 ± 0.4
	P-10	27 ± 2	0.5 ± 0.1	129	94	96	90	24 ± 1
	P-11	25 ± 3	0.5 ± 0.1	126	93	98	91	23 ± 3
^a Results	expressed as	s mean value	e + S.D. (n = 3)	3). ^b Values pre	eviously reported by Rene	edo et al. ³⁸ ^c Chick.		

°C; a source heater temperature of 120 °C; a nitrogen sheath gas flow of 15, an auxiliary gas flow of 5, and a sweep gas of 1 (arbitrary units); and an S-lens RF level of 95%. Mass spectrometer calibration was performed with a mixture of caffeine (m/z 195.08765), Met-Arg-Phe-Ala (MRFA) (m/z 524.26499), and Ultramark polymer (m/z 1221.99063) dissolved in 50% acetonitrile and 0.1% formic acid solution. Mass spectral data were processed with Xcalibur 2.1 software (Thermo Fisher). In full scan mode, an m/z range of 120–1800 was scanned for the detection of Se biocompounds at a resolution of 100 000 ($m/\Delta m$, FWHM at m/z 400). The Se isotopic patterns were searched using Thermo MetWorks 1.2.1 software.

RESULTS AND DISCUSSION

Se Concentrations in the Blood and Internal Tissues. The total Se concentrations determined in the internal tissues and blood of eight giant petrels are presented in Table 1. Despite the high variability in the Se concentrations found in the analyzed samples, there is a clear trend of the highest bioaccumulation of Se in the liver. The hepatic Se concentrations range between 19 and 426 μ g g⁻¹ dw. Se concentrations in kidneys are in general slightly lower than those in the liver, followed by the brain, blood, and muscles. A similar trend was reported in other procellariiform seabirds.^{26,42,43}

Se concentrations in all of the tissues of the single chick are much lower than those in the adult birds, with, for example, only 19 μ g g⁻¹ in the liver (i.e., around 5–20 times lower than those in adult birds). The increase of Se content in internal tissues with age has been previously reported in other (marine) animal species,⁴⁴ which is attributed to the bioaccumulation of

this element during their lifespan.^{45–48} The principal source of Se in birds is dietary exposure.^{14,49} Specifically, in the case of chicks, the Se source is probably a mixture of dietary exposure during the chick-rearing period and the initial maternal Se transfer in the egg.^{24,50,51}

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Se Species Screening in the Water-Soluble Fraction. Se species in the water-soluble fraction were initially screened in a size-exclusion chromatographic column (Superdex 200), with a linear separation range between 3 and 600 kDa. The obtained chromatograms (Figure 1) reveal that Se elutes in the main fraction at approximately 30 min and therefore in the low-molecular-weight range. Interestingly, a similar profile is observed in all of the internal tissues (liver, kidneys, muscle, brain, and testicles) and also in the blood.

Considering that Se in the water-soluble fraction from the different tissues is principally found at a low molecular weight, an additional column (Superdex peptide) with a better resolution (operational separation range of 0.1-7 kDa) was used. The obtained chromatograms (Figure S1) revealed once again the presence of a major Se fraction, eluting at 27 min in all of the samples.

To determine the identity of the observed Se-containing fraction, an additional hyphenated approach was adopted through the coupling of HILIC to ICP-MS and ESI-MS (Figure 2). A single Se-containing peak, eluting at 21 min, was observed. The spectra obtained by HILIC-ESI-MS at the mentioned retention time revealed the presence of a unique Se isotopic pattern at m/z 278.04065, corresponding to selenoneine (2-selenyl- $N\alpha$, $N\alpha$, $N\alpha$ -trimethyl-L-histidine). In addition, the same samples were reduced with TCEP and derivatized with iodoacetamide, as detailed by Klein et al.,⁴⁰ before analysis to check for the potential presence of additional



Figure 1. Typical SEC₂₀₀-ICP-MS chromatograms of ⁸²Se (solid line) and ²⁰²Hg (dotted line) corresponding to the water-soluble fraction of the (a) blood, (b) brain, (c) liver, (d) kidneys, and (e) muscles of adult giant petrels. The inset shows the corresponding zoomed-in views.

selenoneine bound to high-molecular-weight fractions (e.g., bound to protein thiols). However, no significant differences were observed compared to underivatized samples (data not shown). Derivatization was also tested before and after extraction, and no significant differences were detected. In the investigated set of samples, the dimer of selenoneine and the methylated form of this Se species, methylselenoneine, were not found.

Selenoneine, a selenoamino acid that was reported for the first time in 2010,⁵² has been identified so far in a reduced number of samples. Selenoneine has been reported in different biological matrices of animal origin, precisely in the liver of sea turtles⁵³ and dolphins,³⁹ in the blood, liver, spleen, heart, and muscles of several fishes (i.e., Pacific mackerel, tilapia, sardine, and tuna), chicken liver, squid digestive gland,^{52,54} beluga skin,⁵⁵ and human blood^{40,55–57} and urine.⁴⁰ To the best of

our knowledge, this is the first study that identifies selenoneine in seabirds and, more generally, in organs like testicles and the brain.

Considering that a single Se fraction was found in both sizeexclusion chromatographic columns (Figures 1 and S1) and also in the specific analyses by HILIC-ICP/ESI-MS/MS (Figure 2), the proportion of Se present as selenoneine (Table 1) was estimated by calculating the percentage of the peak area in the size-exclusion chromatogram, after verification of quantitative Se recovery from the (Superdex 200) chromatographic column (details in Table S5). It should be highlighted that the reported values correspond to an estimation of the content of selenoneine used for comparison between the birds from this set of samples. Quantification approaches by postcolumn isotopic dilution⁵⁷ and/or external calibration⁵⁵ would provide more accurate values. It should be



Figure 2. Liver extract after acetonitrile treatment analyzed by (a) HILIC-ICP-MS (m/z ⁸²Se) and HILIC-ESI-MS; (b) mass spectrum obtained at 19.8 min, and (e) extracted ion chromatogram of m/z 278.0407 ± 0.02. Insets: (c) corresponding zoomed-in view of mass spectra showing the isotopic pattern of Se obtained by ESI-MS for the selenoneine ion and (d) the theoretical isotopic pattern of selenoneine ion ($C_9H_{16}N_3O_2Se^+$).

mentioned that, so far, there is no commercial standard of selenoneine. The mean percentage of Se as selenoneine in the

water-soluble fraction of the set of samples analyzed was $93 \pm 7\%$ (Table 1). Among the different tissues, the highest



Figure 3. SEC₂₀₀-ICP-MS chromatograms (⁸²Se) corresponding to the water-soluble fraction of blood of chick (solid line) and adult (dotted line) giant petrels.

concentration of selenoneine was found in the kidneys (88 μ g Se g⁻¹).

Even though the age of all of the birds could not be precisely estimated, P-P1 was a chick, while P-10 and P-11 were adult birds. The analyses of the tissues from these birds, in which blood and internal organs were sampled, clearly show a noticeable increase of selenoneine from chick to adults (Table 1). Only one chick was investigated in this work; therefore, the conclusions of this study should not be extrapolated. However, considering that, so far, there are no studies on birds of different ages and the rarity of this kind of sample from remote areas, it is worthwhile to present the results of this preliminary comparison. Unequivocally, definitive conclusions on the effect of age require a study with a larger population.

The highest variation with age was observed in blood, and adult birds presented values around 20 times higher than that in the chick. The Se chromatograms corresponding to the water-soluble fraction clearly show the effect of age on increased Se levels (Figure 3 and Table 1). In the case of chick's blood (Figure 3), some Se-containing fractions were observed at a high molecular weight (HMW), eluting around 10 and 22 min, whereas selenoneine exhibits the lowest percentage of all of the analyzed samples, i.e., 76% (Table 1). In adult birds, the intensity of the Se fraction at HMW remains similar, while a significant increase of the peak corresponding to selenoneine was observed (Figure 3). The Se compounds eluting between 10 and 22 min probably included selenoproteins that are known to play key physiological roles, like selenoprotein P, glutathione peroxidase, and/or seleniumcontaining proteins such as selenoalbumin,^{57–59} among others. Interestingly, the intensity of the mentioned Se-proteins was maintained at similar levels in chicks and adults, suggesting their regulation and essential implication in metabolism. Considering that blood reflects the recent dietary uptake,^{60,61} the differences observed between selenoneine levels in chicks and adults could be attributed to the divergent dietary sources according to the animals' age. It should be highlighted that the investigated set of samples, in terms of the comparison of adults and chicks, is quite limited. The results discussed should, therefore, be considered preliminary. In addition, the Se profile specifically observed in the chick could also be a consequence of the emaciation of this individual. Further studies are required to precisely know whether the presence of selenoneine in the blood is also a result of internal organ redistribution.

Only a few studies reported on the presence of selenoneine in biota, and there is a lack of information about the sources of this selenocompound. Dietary exposure seems to be the principal source of selenoneine for animals.^{40,56,62} The bioaccumulation pathway of selenoneine is expected to be similar to that of ergothioneine (its sulfur analogue) based on their chemical similarity.⁶⁸ Ergothioneine is primarily synthesized by fungal and bacterial species^{63–65} and largely accumulated in animals through the food chain.⁶⁶

The identification of selenoneine in the blood of Inuits from Nunavik is one of the key studies that support the trophic transfer of this selenocompound.^{55,57} Selenoneine is bioaccumulated in this human population through beluga mattaaq consumption, a delicacy highly praised by Inuits, consisting of the skin with the underlying layer of fat, where selenoneine represents up to 74% of the total Se content.⁵⁵ Se speciation in the liver of turtles revealed that selenoneine is exclusively found in marine species but not detected in terrestrial species,⁵³ which has been attributed to the trophic transfer through the marine food webs.^{54,56,67}

The comparison of selenoneine content in the tissues and blood of giant petrels (Table 1) supports its trophic transfer in marine ecosystems. Giant petrels, being top predators, could bioaccumulate selenoneine from their large variety of prey that comprises fish, other seabirds, and stranded marine mammals.^{23,68–71} The levels of selenoneine found in giant petrels are the highest reported in animal tissues until now, with values reaching up to 88 μ g Se g⁻¹ (Table 1).

The current work reports for the first time a potential increase of selenoneine concentration from juvenile (chicks) to adult birds. The highest values found in adults could be justified by their dietary intake. Several studies report an increase of ergothioneine with age until adulthood, followed by a decrease with aging (senescence).⁷²

The brain exhibits the highest fraction of Se as selenoneine of all internal organs. The percentage of selenoneine, comprising the chick and two adults, is quite homogeneous in this organ (78–88%), suggesting that selenoneine plays a crucial role in the protection/functioning of the nervous system. The functions of Se in the brain are considered vital,^{73–75} and its homeostasis in this organ has been evidenced in several studies.^{73,75} The deficiency of this element leads,



Figure 4. Relationship between the selenoneine fraction and Hg concentration in (a) the liver (with a logarithmic curve) and (b) brain of giant petrels.

among others, to irreversible neuronal tissue injury, causing several diseases.^{74–76} The transfer of selenoneine in a well-established in vitro model of the blood–brain barrier has been recently demonstrated,⁷⁷ and our study reveals its presence in this organ for the first time.

In humans, the enhancement effects on cognitive function associated with an increase in mushroom consumption evidenced the crucial role of ergothioneine in the brain.⁷⁸ Several studies demonstrated that this compound could promote neurogenesis and act against oxidative damage and neuroinflammation.^{72,79–81} The precise role of selenoneine in this organ is not known so far. However, considering its similarity with ergothioneine, already characterized in the brain, the role of selenoneine could be related to the protective and antioxidant effect in the nervous system.

Potential Role of Selenoneine in (Methyl)mercury Detoxification. As previously mentioned, the identification of selenoneine in biota is relatively recent, and the metabolic mechanisms in which this compound is involved remain unknown. Some studies reported a potential role of this selenoamino acid in the detoxification of MeHg;^{39,55,57,62,82} however, the mechanism has not yet been characterized. In vitro studies on zebrafish embryos evidenced a decrease of both MeHg accumulation and toxicity in the presence of selenoneine and a selenoneine-specific transporter, organic cations/carnitine transporter-1 (OCTN1).⁶² The hypothesis is that selenoneine plays a protective role through a direct conjugation with MeHg, leading to HgSe formation.⁶² Human studies also speculate about the involvement of selenoneine in MeHg detoxification.^{55,57} Selenoneine is the major Se species in red blood cells of the Nunavut Inuit population and has been related to MeHg demethylation in blood, potentially reducing its distribution to other organs.⁵⁵

HgSe nanoparticles have been recently identified in several organs of giant petrels, including the kidneys, muscles, brain and liver representing up to 95% of the total Hg in the latter tissue.²⁶ In addition to tiemannite, another Hg-Se complex, a tetraselenolate complex with a molar ratio of 1:4 (Hg/Se), was reported in these samples.²⁶ According to Palmer et al.,⁸² MeHg demethylations could be mediated by a structural analogue of selenoneine (1-methyl-1,3-dihydro-2H-benzimidazole-2-selone) through a Hg-Se complex exhibiting a similar Hg/Se ratio (1:4). The selenoamino acid complexes readily promote MeHg-induced proteolytic cleavage of Hg-C bonds.⁸² Therefore, considering that (i) selenoneine is the major water-soluble Se species in giant petrel samples and that (ii) the coordination number of Hg with selenoneine is four,⁸² we could speculate that the identified selenoamino acid is directly involved in the MeHg demethylation step prior to HgSe precipitation.

Laboratory experiments with a variety of selenoamino acids, such as selenocysteine, selenoglutathione, selenopenicillamine, and selenomethionine, already demonstrated their chemical MeHg demethylation capabilities through the formation of unstable complexes before HgSe precipitation.^{83,84} A remarkable decrease in the proportion of Se as selenoneine with increasing liver Hg content was found in giant petrels (Figure 4a).

The decrease in the selenoneine proportions (from 70 to 3%) with increasing Hg concentrations could be a consequence of the implication of this selenoamino acid in the demethylation of MeHg, leading to the codegradation of MeHg and selenoneine, resulting in HgSe formation, the main Hg compound (95%) in the liver.²⁶ In contrast, the percentage of selenoneine in the brain remains stable irrespective of the increase of Hg concentrations (Figure 4b). HgSe nanoparticles are also formed in the brain (approximately 35% of total Hg)²⁶ as a result of MeHg detoxification. Considering that this organ shows the highest Se/Hg molar ratio (33–77) and percentage of selenoneine (80%), we hypothesize that this novel selenocompound plays a crucial role in the nervous system, probably playing a protective role against Hg toxicity.

In summary, the main outcome of this study is the identification of selenoneine in seabirds for the first time. This relatively novel selenocompound represents the main Se species in the water-extractable fraction in all giant petrel samples studied (liver, kidneys, blood, brain, and muscles). The relevance of this compound in biota is still unknown; our work encourages urgent studies of larger animal populations to investigate aspects such as the variation of selenoneine content with age and its potential role in MeHg demethylation. Selenoneine characterization of seabird prey represents another perspective of the present study, which should shed light on the trophic transfer process of selenospecies.

ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c04966.

Details of sampling data set; a general method used for the complete digestion of biological samples; experimental parameters for total Se and speciation analysis by ICP-MS and HPLC-ICP-MS; concentrations of total Se and Hg concentrations in certified reference materials analyzed for quality control purposes; and estimation of the proportion of selenoneine in the samples (PDF)

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Notes

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