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Applied methodology

Determination of selenium and its compounds in marine organisms☆

Małgorzata Anita Bryszewska[a,*], Amund Måge[b]

[a] Wydzial Biotechnologii i Nauk o Zywnosci, Politechnika Lodzka, ul. Stefanowskiego 4/10, 90-924 Lodz, Poland
[b] Nasjonalt Institutt For Ernaerings- og sjoematforskning, Postboks 2029 Nordnes, 5817 Bergen, Norway

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ABSTRACT

In this study, we investigate the type and quantity of selenium compounds in fish and marine organisms, using ion-pair reversed phase LC–ICP-MS, developed and applied for the analysis of Atlantic cod, Atlantic salmon, Greenland halibut, Atlantic herring, blue mussel, common crab, scallop, calanus, and *Euphasia super*. Of the samples examined, the lowest level of selenium was found in farmed Atlantic salmon (0.17 mg Se kg^{-1} dm). The total selenium extraction efficiency by phosphate buffer was 2.5 times higher in sea plankton and shellfish samples than in fish samples. Analysis of Se species in each hydrolysate obtained by proteolysis showed the presence of selenomethionine, which constituted 41.5% of the selenium compounds detected in hydrolysates of Atlantic herring and 98.4% of those in extracts of Atlantic salmon. Inorganic compounds, such as selenates and selenites, were detected mainly in sea plankton and shellfish samples (<0.13 mg Se kg^{-1} wm), although no correlation was found between the presence of inorganic compounds and total selenium concentration. The accuracy of the total selenium determination was validated using a certified reference material (oyster tissue (NIST 1566b)). A lyophilised powder of cod (*Gadus morhua*) was used to validate speciation analysis, enzymatic hydrolysis of lyophilised powder of cod recovered 54 ± 6% of total selenium, and SeMet constituted 83.5 ± 5.28% of selenium detected in hydrolysates. The chromatographic detection limits were, respectively, 0.30 ng mL^{-1}, 0.43 ng mL^{-1}, 0.54 ng mL^{-1}, 0.55 ng mL^{-1}, 0.57 ng mL^{-1} and 0.72 ng mL^{-1} for selenate, selenomethionine, selenite, Se-methyl-selenocysteine, selenocystine and selenomethionine selenoxide.

The data on selenium concentrations and speciation presented here could be useful in estimating levels of selenium intake by seafood consumption.

Introduction

Selenium (Se) is a minor but essential dietary element for both humans and animals, including fish. In the case of fish, Se essentiality and requirements have been estimated for: rainbow trout [1], channel catfish [2], grouper [3] and yellowtail kingfish [4]. Se functions are mediated by selenoproteins. Aquatic organisms (fish and some algae) contain the largest selenoproteomes of any organisms, including mammals [5,6]. Se-containing proteins and enzymes are required for functions such as antioxidant defence, reducing inflammation, production of thyroid hormones, DNA synthesis, fertility and reproduction [7–9]. More recently, evidence has suggested that selenium deficiency could lead to diabetes mellitus and cause atherosclerosis in diabetic patients [10–12]. Se intake varies widely in different areas of the world, due to variations in diets such as protein content, extent and type of food processing and Se concentrations in local food products. The large number of factors that can affect Se levels in foods mean that Se intake differs not only between countries but also between regions [9,13]. Depending on where and how it was produced, there can be up to a 10-fold difference in the Se content of certain foods. The range of mean Se contents in Irish bread, for instance, is 15–158 μg/kg [14].

Se intake by adults has been reported as ranging in Europe from 30 to 100 μg day^{-1} [8,15], in North America from 60 to 220 μg day^{-1} and among some populations in New Zealand from 19 to 80 μg day^{-1}. The European population reference intake for individual males aged 18 years and over (PRI, Scientific Committee for Food, 1992) [16,17] and the recommended dietary allowance in the USA and Canada is set at 55 μg day^{-1}. In Australia and New Zealand, the recommended dietary intakes (RDI) for adult males and females are 70 μg day^{-1} and 60 μg day^{-1}, respectively [18]. These recommendations do not consider the chemical speciation of the element, however [19]. A report by the US Food and Nutrition

☆ Laboratory in which the work was carried out: National Institute of Nutrition and Seafood Research.

* Corresponding author. Tel.: +48 426313425.
E-mail addresses: malgorzata.bryszewska@p.lodz.pl (M.A. Bryszewska), Amund.Maage@nifes.no (A. Måge).

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Board in 2000 suggests that more than 90% of selenomethionine (SeMet) can be absorbed, whereas approximately 100% of selenate is absorbed, although a significant fraction is lost in the urine. More than 50% of selenite is absorbed and is better retained than selenate [20].

In cereals, selenium concentrations vary from 0.01 to 0.55 $\mu g\,g^{-1}$ fresh mass and SeMet is the predominant form, accounting for around 60% of total selenium [21]. Most Se in mussels and fish has been associated with organic forms of the element such as selenomethionine [22].

The aim of this study was to investigate the quantity and type of selenium compounds in fish and other marine organisms. The results could be useful in evaluating dietary information concerning Se content in seafood.

Materials and methods

Standards and chemicals

All chemicals used were analytical grade or above. High-purity deionised water was used throughout the experimental work (18 MΩ, Milli-Q Element system, Millipore, Bedford MA, USA). The following selenium standards were used: seleno-DL-methionine (SeMet, ≥99% purity, Sigma, Oslo, Norway), sodium selenate (Se(VI), ≥98% purity, Sigma, Oslo, Norway), sodium selenite (Se(IV), 99% purity, Sigma, Oslo, Norway), seleno-L-cystine ($SeCys_2$, 99.5%, Aldrich, Deventer, Holland), Se-methyl-seleno-L-cysteine (SeMetCys, ≥98% purity, Fluka, Oslo, Norway). Selenomethionine selenoxide (SeOMet) was prepared in the laboratory according to the procedure described by Fang et al. [23]. Stock solutions of standards were prepared in water in concentrations of one gram of selenium per litre of solution and stored at 4 °C until used. Working solutions were prepared daily.

The mobile phases used for liquid chromatography (LC) are described in Table 1. The reagents for mobile phases included: dibasic ammonium phosphate (Sigma–Aldrich, Oslo, Norway), ammonium sulphate (Sigma–Aldrich, Oslo, Norway), tetrabutylammonium hydroxide (TBA, 40% solution Sigma–Aldrich, Oslo, Norway), and tetraethylammonium (TEA, 35% solution, Alfa Aesar, Karlsruhe, Germany). The buffer solution pH was adjusted by adding either phosphoric acid (crystalline, ≥99,999%, Aldrich, Sigma–Aldrich, Oslo, Norway,) or ammonium hydroxide (28% aqueous solution, ≥99.99%, Sigma–Aldrich, Oslo, Norway).

Table 1
Instrumental settings for Agilent 7500c ICP-MS and chromatography conditions.

ICP-MS settings	
RF power	1550 W
Carrier gas flow	1.2 L/min
Plasma gas flow	15 L/min
Auxiliary gas flow	1.0 L/min
H_2 gas flow (for collision-reaction cell)	3.5 mL min^{-1}
Nebuliser	Babington
Spray chamber	Water cooled double pass
Spray chamber temperature	2 °C
Interface cones	Platinum
Lens voltage	2–3 V
Mass resolution	0.8 u
Integration time	1000 s
Isotope monitored	^{78}Se, ^{80}Se, ^{82}Se
Chromatography	
Injection volume	40 μL
Flow rate	1 mL min^{-1}
Mobile phase	A: 2.5 mmol dm^{-3} $(NH_4)_2PO_4$; 5 mmol dm^{-3} TBA (tetrabutylammonium hydroxide); 3% methanol (v/v); pH 6.5 B: 5 mmol dm^{-3} $(NH_4)_2SO_4$; 3% methanol (v/v); pH 6.5
Separation mode	Gradient mode: 0–6 min: 100% A 6–6.5 min: change to B with linear slope 6.5–17.5 min: 100% B 17.5–18 min: change to A with linear slope 18: 100% A

Samples

The samples submitted to the laboratory were either frozen muscle tissue from five fish, or single fish stored on ice. All samples were obtained from the National Institute of Nutrition and Seafood Research (Bergen, Norway), and had been collected from various locations along the Norwegian coastline as part of a Norwegian monitoring programme on undesirable substances. Collection, selection and preparation of fish samples were performed as part of the scheme set up by the Norwegian Food Safety Authority [24,25], to monitor the presence of group A components (hormone-like substances, steroids, illegal drugs) or group B components (antibacterial agents, anthelmintics, organochorine compounds organophosphorous compounds, heavy metals, mycotoxins). The samples submitted to the laboratory were either frozen muscle tissue from five fish, or single fish stored on ice. On arrival to NIFES, the fillets or chops from the five-fish samples were homogenised into pooled samples with equal mass contributions from each of the fish, and the pooled sample was then frozen. Samples of the shellfish and sea plankton were also homogenised and freeze-dried. Homogenate samples were prepared from: cod (*Gadus morhua*), Atlantic salmon (*Salmo Salar*), Greenland halibut (*Reinhardtius hippoglossoides*), Atlantic herring (*Clupea harengus*), blue mussel (*Mytilus edulis*), common crab (*Cancer pagurus*), scallop, calanus (*Calanus finmarchicus*) and *Euphasia superb.* The analyses were conducted in 2009 and 2010.

Analysis of samples

Total selenium content

To determine the total content of Se, approximately 0.2 g of a solid sample or 1.5–2 mL of extract (an extract after extraction with phosphate buffer) or a hydrolysate (a protease hydrolysate) was digested using a Milestone microwave laboratory digestion system MLS-1200 MEGA (Milestone, Sorisole, Italy) in the presence of 2.0 mL concentrated nitric acid (HNO_3, 65%, Suprapur, Merck, Darmstadt, Germany) and 0.50 mL hydrogen peroxide (30%, w/w, ISO, Merck, Darmstadt, Germany). The sample digests were diluted with MilliQ water to a final volume of 25 mL. This procedure was performed in triplicate for each sample. Three reagent blanks and two samples of a standard reference material of oyster tissue (NIST 1566b, National Institute of Standards and Technology, Gaithersburg, MD, USA) undergo the sample preparation procedure and were included in each series of sample measurements.

Extraction of selenium compounds

Selenium compounds were extracted from the samples by enzymatic hydrolysis using a non-specific protease (Protease type XIV from *Streptomyces griseus* EC 3.4.24.31). Frozen samples were allowed to thaw in a refrigerator (8 °C). Lyophilised samples did not require any pre-treatment. Approximately 0.2 g of each sample was suspended in 2.5 mL of ammonium phosphate buffer (1 mM) containing the enzyme (8 g L^{-1}) and incubated in a tube rotator for 24 h at room temperature (21 °C). A simple extraction of the phosphate buffer was performed as a procedural blank. All the extraction and proteolysis procedures were performed in triplicate. For each experiment, two samples of lyophilised powder of cod (*G. morhua*),