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Analytical methodology

Arsenolipids show different profiles in muscle tissues of four commercial fish species

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ABSTRACT

Identification of arsenolipids in biological samples is today a challenge and in particular the need for speciation data for toxicological assessment. Fish is one of the major contributors of arsenic in diet. However, the majority of work in this area has only focused on the water soluble compounds. The aim of this study is to provide some data on total arsenic and in particular to gain insights into the types of arsenolipids in the muscle tissues of four commercial and commonly consumed fish species. Determination of total arsenic was carried out by ICP-MS following microwave-assisted acid digestion of the samples and the concentrations found for total arsenic in the muscles ranged from 4.8 to 6.0 μg/g d.w. Sequential extraction was carried out using hexane and MeOH/DCM followed by reversed phase HPLC-ICP-MS/ESI-MS analysis of the MeOH/DCM fraction. Eight arsenolipids including three arsenic fatty acids (AsFAs) and five arsenic hydrocarbons (AsHCs) were identified. The result showed that fish with higher arsenolipid (AsLp) content (brill and sardine) are dominated by AsHC, while those with the smaller proportion of AsLp (mackerel and red mullet) have predominately arsenic in the form of AsFA.

Introduction

The study of arsenic in aquatic environment is essential to understanding its global cycling and biotransformation [1]. In seawater, arsenic is usually present at low concentrations typically 1–2 μg/L [2–4]. On the other hand, marine organisms are capable of bioaccumulation [5] and contain high concentrations of arsenic, usually 5–100 mg/kg d.w. [6]. Arsenate being the predominant form in seawater is readily taken up by alga from the surrounding water. It is speculated that this adsorption is due to the inability of phosphate transporters to differentiate between the structurally similar arsenate and phosphate in the marine environment [4,7]. As part of the detoxification process, the arsenic taken up from the marine environment undergoes a series of biotransformations in the organisms resulting in a wide range of organoarsenic compounds [8].

Marine animals accumulate arsenic mainly from their food, however, like algae fish are capable of synthesizing both lipid-soluble (arsenolipids) and water-soluble organo arsenic compounds [9,10]. Furthermore, it has been established that arsenobetaine (AB) and arsenosugars (dimethylarsinoylribosides) are predominant in marine animals and marine algae respectively. Other methylated water-soluble arsenic compounds, such as methylarsonic acid (MA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), and tetramethylarsonium ion (TMA), are also widely found in marine organisms mostly as minor constituents [11]. Inorganic arsenic comprising arsenite and arsenate, have been found in fish and shellfish, but constitute only a few per cent of the total arsenic content [12]. The majority of these studies have only investigated the water soluble compounds comprising over 80% of non-toxic arsenobetaine in most cases [3,13]. Though the highly toxic inorganic arsenic content in fish is usually very low, at the same time, it will be wrong to assume that the arsenic in fish and other seafoods are non-toxic. Apart from non-toxic arsenobetaine, arsenosugars and arsenolipids found in large amount in marine samples undergo biotransformation to produce potentially toxic intermediates and some common metabolites with inorganic arsenic including dimethylarsinic acid (DMA) and methylarsonic acid (MA) [14–16]. Since the toxicity of arsenic depends on the chemical forms, it is important to identify the chemical species and amounts of arsenolipids in marine samples.

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In the last couple of years, a number of arsenolipids have been identified including AsFAs, AsHCs, arsenosugar phospholipids (AsPL) and trimethylarsenio fatty alcohols (TMAsFOH) [17–20]. Though fish muscle is an essential and common constituent of the diet, very little is known regarding the arsenolipid contents distribution. Therefore, the present study investigates the comparative study of the arsenolipids profiles in muscle tissues of four commonly consumed and commercial fish species.

Experimental

Reagents and chemicals

Ultra-pure water (18 MΩ cm Elga, UK) was used throughout for sample preparation. Formic acid and sodium arsenite were supplied by Sigma–Aldrich (UK). Sodium dimethylarsinic acid (98%, DMA^V), used as calibration standard for quantification of arsenic species was obtained from ChemService (USA). Hexane, hydrogen peroxide (H_2O_2, 32%), dichloromethane (DCM) and methanol were obtained from Fisher Scientific (UK). Nitric acid (HNO_3, 65%) from Fluka (UK) and the certified reference material used was DORM-2 and DORM-3 (NRCC).

Samples and sample preparation

The samples of fish analyzed in this study include sardine (*Sardina pilchardus*), mackerel (*Scombers combrus*), red mullet (*Mullussur muletus*) and brill (*Scophthalmus rhombus*), and were purchased from local fish market in Aberdeen, UK. For each fish sample, only the muscle tissue was taken for analysis. The samples were freeze dried to a constant weight and then pulverized by grinding in liquid nitrogen with mortar and pestle. The four species of fish analyzed include two specimen of sardine (1 and 2) and three samples of mackerel (1–3). Triplicate analysis was carried out on individual samples and sample extracts for total arsenic and the result expressed as mean value (X) ± standard deviation (SD).

Sequential solvent extraction procedures

About 1–2 g of freeze dried fish muscles were first extracted with 20 mL of hexane. Each fish sample was shaken overnight with extracting solvent at room temperature and then centrifuged at 3500 rpm for 15 min. After the hexane extract was removed, the residues was further extracted twice using 20 mL methanol/DCM (1:2) for the polar arsenolipids. Total concentrations of arsenic in the extracts were determined directly. But only the methanol/DCM (1:2) fractions were further investigated for arsenolipid speciation.

Determination of total arsenic by acid digestion and ICP-MS

The total arsenic content was established in the freeze dried tissue samples and sample extracts using an Agilent 7500c ICP-MS (Agilent Technologies, California, USA) following acid and microwave digestion. About 0.1–0.5 g of the samples were weighed into 50 mL plastic vials and 1 mL of conc. HNO_3 added and left overnight, 2 mL of H_2O_2 was then added and the vials placed in a microwave digestion system (Mars-5, CEM, UK.) for 40 min with the temperature ramped up to 90 °C. After cooling the digests were diluted to 10 mL and total arsenic concentration determined, taking into account the change in density. ^{74}Ge was used as internal standard and quantification was carried out against standard solutions of sodium arsenite. Arsenic was measured at *m*/*z* 75. Se was monitored at *m*/*z* 77 and 82 for the correction of possible interference by $ArCl^+$. But no correction was necessary as chloride interference was not detected. The analytical method was validated by the measurement of certified material DORM-3 with the certified value of 6.88 ± 0.30 μg As/g and DORM-2 with certified value 18.00 ± 1.1 μg As/g. The determined values for total arsenic in DORM-3 (6.85 ± 0.22 μg As/g) and in DORM-2 (17.66 ± 0.14 μg As/g) are in good agreement with the certified values. All measurements for total arsenic concentrations were carried out in triplicate and results were expressed as (X ±SD).

Table 1
HPLC-ICP-MS/ESI-MS parameters.

HPLC	Thermo Scientific
Column	Agilent Eclipse, XBD-C18; 4.8 mm × 150 mm
Column temperature	30 °C
Injection volume	100 μL
Buffer A	0.1% formic acid in water
Buffer B	0.1% formic acid in methanol
Splitter ratio	1:3
Flow rate	1 mL/min
Gradient	0–25 min: 0–100%, 5 min 100% B
ICPMS	Element 2 (Thermo Scientific)
Mode	Organic mode
HF	1370 W
Nebulizer	Micro-concentric
Nebulizer gas	0.86 L/min
Optional gas	20 mL/min O_2
Plasma gas	0.89 L/min
Coolant gas	14.9 L/min
ESI-MS	LTQ Orbitrap Discovery; Thermo Scientific
Mode	Positive
Spray voltage	4.5 kV
Normalized collision energy	35%

Speciation analysis

Identification of arsenolipids

Speciation of arsenolipids was carried out by reversed phase HPLC on-line with ICP-MS and ESI-Orbitrap-MS as previously described [21]. The arsenic species were separated using a gradient of 0.1% formic acid in water and 0.1% formic acid in methanol on a reversed phase column, Agilent Eclipse XBD-C18 (4.6 mm × 150 mm) with a flow rate of 1 mL/min, injection volume of 100 μL and column temperature of 30 °C. The eluent was split post column with 25% going into the high resolution ICP-MS (Element 2, Thermo Fisher) and the 75% to Electrospray Ionization Orbitrap Mass Spectrometer (ESI-Orbitrap-MS). The Orbitrap was used in positive scan mode. The ICP-MS was used in low resolution with platinum cones and optional gas flow of O_2 at 20 mL/min. The ICP-MS signal was optimized to give a maximum response of As signal at *m*/*z* 75. DMA^V was used as element specific standard for external calibration and quantification of the arsenic species by ICP-MS with ^{74}Ge as internal standard to monitor the stability of the plasma (Table 1).

Results and discussion

Total arsenic in fish and fish extracts

The total arsenic concentrations in dry weight (d.w) in the muscle of the four species of fish ranged from 4.9 to 6.0 μg As/g (Table 2). The concentrations obtained for total arsenic are within the normal range commonly reported for arsenic in marine organisms [6]. No significant inter- and intra-species variation was observed among the fish investigated. However, due to the small number of samples it is not possible to make a general statement about the variability in the concentration of total arsenic. Fractionation through sequential extraction was further investigated in addition to a detailed analysis of the molecular forms of the arsenolipids.The sequential extraction procedure involved two steps which produced the non polar arsenolipids in the hexane fraction and the