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The role of metallothionein and selenium in metal detoxification in the liver of deep-sea fish from the NW Mediterranean Sea

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HIGHLIGHTS

- High levels of some toxic metals are found in NW Mediterranean deep-sea fish.
- Selenium plays a key role in preventing Hg toxicity in deep-sea fish.
- Species exhibited differences in defence mechanisms against metal toxicity.

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ABSTRACT

Seven deep-sea fish species were sampled in the Blanes Canyon area (NW Mediterranean) at a depth of 1200 m during winter. The concentrations of nine metals were determined in the liver of these species by ICP-MS. Furthermore, the metal detoxification potential was determined for each species by analysing the hepatic metallothionein (MT) content, relations between metals and the molar ratio between MT and/or selected metals. The potential effect of metal content on their physiology was assessed using general stress markers such as the enzyme activities of acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) in muscle.
Levels of metals in the seven Mediterranean deep-sea fish species studied were intermediate to equivalent species of fish either from Atlantic waters or hydrothermal vents. The metal detoxification potential varied among species depending on MT, selenium (Se) or zinc (Zn) as reliable mechanisms to handle potential metal toxicity. The role of Se was especially relevant when the liver content of mercury (Hg) was higher. AChE and LDH activities did seem to be affected by metal loads and thus the activities reported would correspond to baseline activities of the selected species.

1. Introduction

The existence of high concentrations of toxic metals, especially mercury (Hg), in some deep-sea environments is well acknowledged (Storelli et al., 2002; Choy et al., 2009). Concerns have been recently raised regarding, in particular, the high levels of Hg in muscle of deep-sea organisms, whose consumption can represent a potential health risk (Drava et al., 2004; Bebianno et al., 2007; Díez, 2009; Koenig et al., 2013a). Previous work has addressed the influence of the geographic area, sampling depth, feeding habits and trophic position on the accumulation of metals by deep-sea organisms (Cronin et al., 1998; Kress et al., 1998; Mormede and Davies, 2001; Martins et al., 2006; Company et al., 2010; Koenig et al., 2013a). Deep-sea fish and, in particular, those inhabiting areas with high presence of metals, essential and non-essential ones, had to evolve to coexist with high concentration of metals without compromising their health status (Company et al., 2010). In addition to the already well known role of the liver in the metabolism of metals, earlier studies on deep-sea fish showed the importance of the liver, in relation to other tissues, in metal storage and also the utility of liver in order to discriminate between populations that are exposed to different levels of metals in their environment (Company et al., 2010). Another key defence mechanism against metal toxicity is the binding of metals to the cytosolic protein metallothionein (MT) of widespread phylogenetic distribution (Viarengo et al., 1999; Wood et al., 2012). In fish, metals such as Ag, Cd, Cu and Zn, but also Hg, are reported to induce the synthesis of MT (Cosson, 1994; Huang et al., 2007; Fernandes et al., 2008; Monteiro et al., 2013).

In the case of Hg, the concomitant presence of Se in tissues seems to play an important role in the storage of Hg and its tissue distribution; thus, providing an important protection mechanism against Hg toxicity (Raymond and Ralston, 2009). Studies on the interaction of Se metabolism and toxicity of Hg compounds have shown that Se protects organisms from the toxicity of both inorganic (Hg) and organic (MeHg) forms

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(Endo et al., 2002; Branco et al., 2012). The high toxicity of Hg, both organic and inorganic, is associated to its high affinity for thiol groups, which causes an efficient binding of Hg to the cysteine residues of many essential proteins and enzymes and therefore affecting their functions (Khan and Wang, 2009). In aid of reducing metal toxicity, the formation of the complex Se–Hg–protein has an important role in preventing the toxicity by inorganic and organic Hg as seen in the liver of marine mammals (Ikemoto et al., 2004). This is due to the fact that Hg has even a greater affinity for Se than it has for sulphur groups of some essential proteins (Wessjohann et al., 2007; Berry and Ralston, 2008). Thus, in order to be able to decrease Hg toxicity, there should be an amount of Se available equal or higher than that of Hg (Raymond and Ralston, 2009). Therefore, if the molar ratio of Se:Hg is greater than 1, it is likely that Se is contributing to the detoxification of Hg (Sørmo et al., 2011). In fact, the ratio Se:Hg in muscle tissue has been established as a good marker to predict the risk for human consumption (Burger et al., 2012). In addition to its protective role as Hg chelator, Se is an essential metal necessary for the proper functioning of enzymes that protect the brain and endocrine tissues against oxidative damage and it is a metal present in some antioxidant enzymes (Livingstone, 2001; Raymond and Ralston, 2009). In aquatic systems, the role of Se in mitigating Hg toxicity has not only been documented in mammals (Cuvin-Aralar and Furness, 1991; Hong et al., 2012), but also in fish (Ordiano-Flores et al., 2012).

The presence of Se in tissues not only reduces Hg but also Cd toxicity by storing and reallocating these toxic metals and thus, diminishing its potential adverse effect. The results from an in vitro study in rats, on the protective role of Se against Cd exposure, suggested that Se produced a Cd–Se complex at first, which subsequently binds to a selenoprotein P, thus reducing Cd availability (Sasakura and Suzuki, 1998). In the same mammalian species, Se also protected in vivo from Cd toxicity (Newairy et al., 2007). To the best of our knowledge, no studies are available on fish. Other transition metals, such as Zn, have also been described to have a protective role against Hg toxicity, at least in mammals (Afonne et al., 2000; Orisakwe et al., 2001). In liver of rat, Zn alone, but more significantly together with Se, efficiently protected them from Cd-induced oxidative stress (Jihen et al., 2009). In fact, a part of the protective role of Se and Zn, in preventing Hg or Cd toxicity is due to their association with antioxidant enzymes (Livingstone, 2001) such as glutathione peroxidase (Se) and superoxide dismutase (Zn).

Muscular enzymes such as acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities have been used in combination as general markers of stress in aquatic organisms, including fish, and have been related to metal exposure in field and lab studies (Wepener et al., 2005; Vieira et al., 2009). AChE acts as a neural transmitter and its activity can be compromised by exposure to many chemicals, mostly pesticides, but also to metals (Gill et al., 1991; Payne et al., 1996; Frasco et al., 2005, 2007). Its inhibition is considered a sign of neurotoxicity. LDH is a key enzyme in the glycolytic cycle; its activity is usually enhanced under stress when there is greater oxygen demand and higher anaerobic oxygen metabolism. Elevated LDH activity levels as response to metal exposure has also been demonstrated in fish (Gill et al., 1990; Vieira et al., 2009).

Due to the lack of knowledge on the mechanisms to deal with high levels of toxic metals of deep-sea fish species, seven benthic fish with a relatively high trophic level ($\delta^{15}N$ between 9.86 and 13.78‰) and belonging to four different phylogenetic families (Polunin et al., 2001; Tecchio et al., 2013) were sampled in the NW Mediterranean. Thus, the aims of the present study were: (1) to study the chemical composition of liver in deep-sea fish from the Blanes Canyon area, in regard to essential and non-essential metals, and to contrast these values with those obtained in the same species, or similar ones, from other geographical regions, (2) to determine MT and Se concentrations to elucidate if there is an indication of their respective protective role against metal toxicity and (3) to combine the use of general stress markers, such as AChE and LDH, as endpoints of metal toxicity in the selected species.

2. Material and methods

2.1. Study area and sample collection

Sampling was carried out onboard the R/V Garcia del Cid in winter (January 2009) at a depth of 1200 m in the Blanes Canyon region (Catalan coast, NW Mediterranean Sea). Coordinates were: 41°15′N 2º50′E. Fish were caught using an OTMS otter trawl and their size, weight and sex were immediately recorded. Liver and also a portion of muscle tissue were dissected, frozen in liquid N_2 and stored at −80 °C for further analysis.

2.2. Analysis of metals

Individual fish liver samples of about 0.1 g wet weight were digested in concentrated nitric acid 65% (Baker) overnight at room temperature and afterwards heated at 80 °C for 2 h. Within each digestion series, appropriate blanks with ultra-pure water were also subjected to the same procedure to account for background contamination levels. After cooling, solutions were transferred to a standard volume with ultra-pure water. Determination of Ag, Cd, Cr, Cu, Fe, Hg, Pb, Se and Zn was undertaken by inductively coupled mass spectrometry (ICP-Mass; Elan DRC-I, Perkin-Elmer Sciex). Samples of similar weight of certified reference material (DOLT-3 and LUTS-1, National Research Council of Canada, Ottawa), were digested and analysed during each analytical run. Results are expressed as $\mu g\ g^{-1}$ wet weight of tissue.

2.3. Determination of metallotionein by differential pulse polarography

About 0.2 g wet weight portions of frozen liver were homogenised using an ultra-turrax in 20 mM Tris–HCl buffer, 1 mM dithiothreitol (DTT) and 0.2 mM phenylmethylsulfonylfluoride (PMSF) at pH 8.6 in an ice bath. The homogenates were centrifuged at 30.000 *g* for 45 min at 4 °C. The resulting supernatant was then heated at 80 °C for 10 min, in order to denature high molecular weight proteins, and subsequently centrifuged at 30.000 *g* for 45 min at 4 °C. This secondary heat-treated supernatant, containing thermally stable MT, was separated from precipitated proteins and used for MT determination. MT was measured using differential pulse polarography as described by Bebianno and Langston (1989). An aliquot of the heat-treated supernatant was added to the polarographic cell, containing 20 ml hexamminecobalt chloride buffer (the supporting electrolyte), together with Triton-X (0.025% v/v). The cell was purged for 2 min with purified N_2 prior to analysis. The polarographic response was measured during a potential scan between −1.38 V and −1.7 V (Model 757 VA Computrace Analyser, Methrom, Switzerland) in SMDE mode. Quantification of MT was performed by using the standard addition method with rabbit liver MT I + II (Sigma). Results are expressed as $\mu g\ g^{-1}$ wet weight of tissue.

2.4. Molar ratios calculations

Molecular ratios were calculated as described in Company et al. (2010). A molecular weight of 6000 Da for MT and the respective atomic weights for metals were adopted for the conversion of MT and metal content (μg/g w.w.) into molar content (mol/g w.w.). In this way, the calculated ratios are expressed as moles of MT per atom of metal in the hepatic tissue.

2.5. Biochemical determinations

A portion of 0.2 g of axial muscle tissue was homogenised in a 50 mM buffer phosphate pH 7.4 in a 1:5 (w:v) ratio using a polytron® blender. The homogenate was centrifuged at 10,000 *g* × 30′ at 4 °C