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A field survey of metal binding to metallothionein and other cytosolic ligands in liver of eels using an on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS)

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ABSTRACT

The effect of metal exposure on the accumulation and cytosolic speciation of metals in livers of wild populations of European eel with special emphasis on metallothioneins (MT) was studied. Four sampling sites in Flanders showing different degrees of heavy metal contamination were selected for this purpose. An on-line isotope dilution method in combination with size exclusion (SE) high pressure liquid chromatography (HPLC) coupled to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS) was used to study the cytosolic speciation of the metals. The distribution of the metals Cd, Cu, Ni, Pb and Zn among cytosolic fractions displayed strong differences. The cytosolic concentration of Cd, Ni and Pb increased proportionally with the total liver levels. However, the cytosolic concentrations of Cu and Zn only increased above a certain liver tissue threshold level. Cd, Cu and Zn, but not Pb and Ni, were largely associated with the MT pool in correspondence with the environmental exposure and liver tissue concentrations. Most of the Pb and Ni and a considerable fraction of Cu and Zn, but not Cd, were associated to High Molecular Weight (HMW) fractions. The relative importance of the Cu and Zn in the HMW fraction decreased with increasing contamination levels while the MT pool became progressively more important. The close relationship between the cytosolic metal load and the total MT levels or the metals bound on the MT pool indicates that the metals, rather than other stress factors, are the major factor determining MT induction.

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1. Introduction

The use of molecular responses, such as metallothionein induction, as biomarkers for the impact of aquatic metal pollution is critically dependent on the understanding of their role in controlling metal metabolism and toxicity (Wallace et al., 2003; Serafim and Bebianno, 2007). Metal toxicity arises predominantly from the binding of metals to essential biomolecules such as enzymes and transporters and the involvement of certain metals in the formation of radicals (Mason and Jenkins, 1995). Protection against metal toxicity can be achieved by the synthesis of metal binding ligands to sequester the incoming metal ions in less reactive pools, including cytosolic proteins like metallothionein (MT), lysosomes and granules (Vijver et al., 2004). This may also influence the subcellular partitioning of essential and non-essential metals and metal-dependent metabolic functions. It is well known that the induction of MT, as response to elevated levels of waterborne and dietary metal exposure, is one of the first and most important responses to fight metal toxicity. MT induction is therefore considered as a potentially powerful biomarker to monitor environmental metal contamination (Olsvik et al., 2001; Perceval et al., 2006). However, metallothioneins are not the only proteins involved in metal binding and it is therefore important to determine which and how much metals are bound to the metallothionein pool in comparison to the other metal binding ligands present.

The coupling of high-resolution techniques, such as HPLC coupled on-line to ICP-MS, provides a powerful tool for this purpose (Goenaga Infante et al., 2006; Santiago-Rivas et al., 2007). The major virtues of these coupled HPLC and ICP-MS techniques are selectivity, sensitivity and multi-element capability. Nevertheless, the lack of standards for the different metal binding proteins present, make on-line quantification of metal species a difficult task. The recent development of an on-line isotope dilution (ID) method in combination with the coupling of size exclusion (SE) HPLC to an inductively coupled plasma time-of-flight mass spectrometer (ICP-TOFMS) makes the on-line multi-element quantitative speciation of metal binding proteins possible (Goenaga Infante et al., 2006). This method has proven to be a powerful technique to perform metal binding and speciation studies of complex mixtures, such as metal binding proteins and natural organic matter (Rottmann and Heumann, 1994; Muniz et al., 2001). So far, the potential of these new coupled analytical techniques has only seen limited environmental and ecotoxicological application (Goenaga Infante et al., 2003; Rodríguez-Cea et al., 2003).

In this work HPLC-ICP-TOF-MS has been used to study the cytosolic fractionation of metals in field populations of the European eel, *Anguilla anguilla*, with special emphasis on the role of metallothionein in metal binding. Yellow eels are bottom dwelling fish which are quite territorial and have a relatively small home-range of around 100–200 m (Slayter, 1981). They feed mostly on benthic invertebrates and are consequently exposed to sediment-associated contamination. They strongly accumulate inorganic and organic pollutants and are therefore interesting organisms for biomonitoring purposes (Sancho et al., 2000; Versonnen et al., 2004). Only little information is available concerning metal accumulation and MT induction in natural populations of the common eel despite the fact that common eels are well spread inhabitants of fresh, estuarine and marine waters and related species occur world wide (Linde et al., 2001; Langston et al., 2002). To investigate the usefulness of metallothionein and other cytosolic metal binding proteins as biomarkers of metal exposure in wild populations of eels the relation between environmental metal exposure, metal accumulation and the metal binding proteins levels was studied. A quantitative analysis of liver cytosolic metal speciation was performed applying on-line isotope dilution with the coupling of size exclusion high performance liquid chromatography (SE-HPLC) to Inductively Coupled Plasma time-of-flight Mass Spectrometry (ICP-TOFMS).

2. Materials and methods

2.1. Sample sites and sample collection

During autumn 2001 (September–November) eels were sampled at four different sites in Flanders using fyke nets. The sampling sites were selected on the basis of data collected in the framework of a monitoring network which included information of metal concentrations in water, sediment and eel muscle tissue from more than 200 sites over Flanders. Three canals were selected: the Beverlo canal as the most polluted site, the Zuid-Willemsvaart as an intermediately polluted site, and the Venepevaart as the least polluted site. As a fourth site lake Weerde was selected with intermediate environmental metal concentrations (Table 1). The effects of environmental factors on the condition of the eels were controlled as much as possible by performing the sampling in the non-migratory period and within a short time frame, by selecting juvenile eels of a similar size, and by reducing the stress of capture and handling as much as possible. The eels were sampled in the yellow pre-migratory stage and not the more mobile silver stage. To exclude effects of body size on MT levels eels of a consistent length (30–50 cm) were sampled. The eels captured at different sites did not differ significantly for their weight nor their length. The eels were directly killed by a blow on the head, weighed and length (±0.1 g) was measured (±1 mm). The livers were excised without rupturing the gall bladder, divided into two parts and separately frozen in liquid nitrogen on site. In the laboratory the samples were stored at −80 °C prior to analysis.

The sampling, digestion and measurement of the total metal concentrations in water and sediment samples were done by the Flemish Environmental Agency (VMM). The water and sediment samples were collected during spring and autumn 2001. The sediment samples were collected using a 'Van Veen' grab sampler, mixed, freeze dried and grinded. To determine the total metal concentrations of the sediments, samples of about 500 mg were transferred to Teflon bombs, to which 4 ml HNO_3 (J.T. Baker Instra Analyzed, Deventer, The Netherlands) and 12 ml HCl (Baker Instra Analyzed) were added and the samples were digested in a pressure-controlled microwave oven (MDS/MARS 5, producer specifications, 2 min at 1200 W, 200 PSI and 10 min at 1200 W, 300 PSI). The water samples were filtered over a 0.45 μm filter and acidified with nitric acid to a 1% nitric acid solution. The metal concentrations in both the water and sediment samples were measured