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Review

How does the metallothionein induction in bivalves meet the criteria for biomarkers of metal exposure?☆

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ABSTRACT

Metallothionein (MT) concentrations in the whole soft tissue or in a particular tissue of bivalves have widely been used in ecotoxicological studies and biomonitoring programmes. This approach is based on the reported results on the enhancement of MT induction in bivalves in response to metal exposure. The validity of using MT induction as a biomarker is briefly assessed in the present study. The sensitivity of MT induction in these organisms is questionable due to the high basal MT level as well as the high natural variability related to the effects of a number of biotic and abiotic factors, which are not well described yet. Moreover, the relationship between exposure to metals, the toxic effects of that exposure, and the appearance of MT in soft tissue, is not well characterized. A variety of factors may influence the appearance and distribution of MT: 1) the uneven distribution of metals in particular portions of the soft tissue and in particular subcellular compartments; 2) pre-exposure to metals, perhaps at non-toxic levels; 3) metal–metal competition and metal-protein interactions; and 4) tissue-specific induction, functions, and isoforms of MT. Therefore, attention is required when using MT induction in bivalves for assessment of metal exposure or consequent toxic effects. The MT concentration can be a reliable indicator only when it is considered in relation with metal uptake kinetics and subcellular partitioning while specifying the isoform of MT synthesised and considering various confounding factors. The kinetic turnover of MT may provide useful information on metal exposure and biological effects since it covers both the synthesis and breakdown of MT as well as the chemical species of metals accumulated and MT.

1. Introduction

Contamination with metals is one of the major concerns for aquatic ecosystems due to their adverse effects on living organisms. Bivalves as active filter-feeding animals have widely been included in biomonitoring programmes as they can accumulate different metals to a high level from water, food, and inorganic particulate material (Phillips, 1977; Goldberg et al., 1978; Otchere, 2003; Rainbow, 2006; Guidi et al., 2010; Le et al., 2011). In aquatic ecosystems, the accumulation of metals in the soft tissues of these filter-feeding bivalves is considered to represent the bioavailable fraction of metals (Walsh and O'Halloran, 1998; Boening, 1999). Moreover, chemical accumulation in bivalves reflects impacts of the chemical exposure on biological processes (Rainbow, 2002). In addition, these organisms have a wide geographical abundance and distribution, relative longevity, and sedentary character while it is easy to collect them and to conduct biochemical and physiological measurements (Santiago-Rivas et al., 2007). Generally, bivalves are able to tolerate fluctuating salinity, temperature, and oxygen contents (Goldberg, 1986). Among others, these characteristics contribute to the wide use of these invertebrates as bioindicators.

Exposure of bivalves to certain metals may lead to changes in biochemical processes that are potential biomarkers of the exposure as well as early warning signals of adverse effects of the metal accumulation within the bivalves. Cytosolic proteins play an important role in the sequestration, elimination, and toxicity of metals to bivalves (Amiard et al., 2006). Metallothionein (MT) synthesis is one of the most important reactions as a response to metal exposure and therefore a potential biomarker for metal uptake (e.g., Lecoeur et al., 2004; Ivankovic et al., 2005; Frank et al., 2008). Metallothioneins have been reported to be induced in different tissues of aquatic molluscs following exposure to various metals (Zorita et al., 2005, 2007; Pytharopoulou et al., 2011; Gillis

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et al., 2014; Chandurvelan et al., 2015). Metallothioneins are low molecular weight, cysteine-rich cytosolic proteins and able to bind to a number of metals, such as Ag, Cd, Co, Cu, Hg, Ni, Pb, Pd, and Zn (Waalkes et al., 1984; Nielson et al., 1985; Hamer, 1986; Kagi and Kojima, 1987; Ng et al., 2007; Frank et al., 2008). The increase in MT concentrations in tissues represents a typical response of bivalves to metal contamination under either field or laboratory conditions (Raspor et al., 1987; Pavicic et al., 1991, 1993; Bebianno and Langston, 1991; Roesijadi, 1993; Bocchetti and Regoli, 2006; Amiard et al., 2006; Serafim and Bebianno, 2007; Viarengo et al., 2007; Frank et al., 2008; Oaten et al., 2015; Geng et al., 2015). In general, MTs are involved in different biological processes, i.e., homeostasis of essential metals, detoxification of toxic metals, and cell protection against oxidative stress (Roesijadi, 1994; Langston et al., 1998; Klaasen et al., 1999; Viarengo et al., 2000; Dabrio et al., 2002; Geffard et al., 2005; Ng et al., 2007). Therefore, MT induction has been included in a number of monitoring programmes and considered in the framework of biological effect quality assurance in monitoring programmes BEQUALM (Mathiessen, 2000; Zorita et al., 2005; Ivankovic et al., 2010) where MT levels are considered to reflect the bioavailability and toxic impacts of metals.

Different definitions have been used for the term “Biomarkers”, but in general, “Biomarkers” are measurements reflecting changes in biological responses at different levels (from molecular, cellular, and individual levels, to populations and ecosystems) following the exposure of organisms to certain stressors (NRC, 1987; WHO, 1993; Peakall, 1994; Van Gestel and Van Brummelen, 1996; Sures et al., 2015). Different from chemical indicators of pollutant contamination in the environment, biomarkers indicate biological effects of pollutants, which are not seen in the non-contaminated environment. Good biomarkers must therefore be sensitive to both pollutant bioavailability and early biological effects (Van der Oost et al., 2003). A number of issues are included in the criteria for biomarkers listed by Van der Oost et al. (2003). These concern the following: 1) availability of reliable, cheap, and low-cost measurements; 2) sensitivity to pollutant exposure and effects; 3) well-defined biomarker basal line in non-contaminated situations; 4) known influence of confounding factors; 5) well-characterised dependence of the response on exposure dose and time; and 6) established relationship between the response and effects on the organism.

Although MT induction in a number of bivalves has been used as a biomarker of metal exposure and effects, variations in MT induction among different metals, organs, species, and exposure scenarios raise questions concerning the validity of this method (see e.g., review by Amiard et al., 2006). Further questions result from the absence of significant enhancement in MT induction in response to metal exposure and insignificant relationships between metal and MT concentrations reported in several studies. In the present study, the use of MT induction as a biomarker of metal exposure and effects was assessed by carefully considering the recognised criteria of a biomarker based on published results. In the present detailed interpretation of previously reported findings, the concentration of induced MT was related to metal uptake kinetics and subcellular partitioning. The induction of MT was additionally evaluated in relation to the induction of different MT isoforms in various tissues and with multiple functions while taking the influence of various confounding factors into consideration. Furthermore, the concentration of MT was considered to be subject to a dynamic balance of synthesis and breakdown of MT.

2. Method for MT determination in bivalves (Criterion 1)

The concentration of MT in tissues of bivalves is usually determined in two steps: isolation of MT and subsequent quantification. The most common isolation methods include solvent precipitation and heat denaturation while the most widely used quantification methods include metal saturation assay, spectrophotometry, and polarography (Piotrowski et al., 1973; Olafson and Sim, 1979; Viarengo et al., 1997; Geret et al., 1998; Petrlova et al., 2007; Frank et al., 2008, 2013). Besides these common methods, a number of others have then been developed and applied to isolate and to quantify MT concentrations in bivalves as reviewed below.

Different methods for isolating cytosolic MT have been investigated. They include using cutting mixers (Roesijadi and Fowler, 1991), rotating pestles and sonication in closed vessels under inert Ar or N_2 atmosphere (Wolf et al., 2002), blending device (Santamaria-Fernandez et al., 2004), centrifugation in combination with heat denaturation or solvent precipitation (Santiago-Rivas et al., 2007), and freeze thaw cycles in liquid nitrogen pressurized liquid extraction (Rudolph et al., 1999; Santiago-Rivas et al., 2007). The pressurized liquid extraction has increasingly been used because of its advantages. Some of the advantages are the possibility for extracting a large number of samples, avoidance of filtration or centrifugation, and small amounts of samples required for the isolation (Santiago-Rivas et al., 2007). In addition, the results generated by this method were compatible to those obtained by using blending devices. Contrasting with this consistency, some differences in the measurements of MT have been shown when different isolation methods were used. For instance, significant differences in MT concentrations were obtained after using the two isolation procedures, heat denaturation and solvent precipitation (Geret et al., 1998), as they act on specific properties of MT isoforms. Similarly, Erk et al. (2002) showed different results on the determination of MT isoforms.

Quantification of MT has been performed on the protein as well as on the mRNA level by a number of methods, e.g., hyphenated techniques (metal saturation assays, chromatography, capillary electrophoresis, mass spectrometry), polarography (electrochemical methods), and immunoassays (Brdicka, 1933; Olafson and Sim, 1979; Lobel and Payne, 1987; Funk et al., 1987; Roesijadi et al., 1988; Chan et al., 1989; Micallef et al., 1992; Hogstrand and Haux, 1992; Livingstone, 1993; Viarengo et al., 1997; Prange et al., 2001; Alvarez-Llamas et al., 2001; Zorita et al., 2005). These methods are based on different properties of MT, e.g., redox properties of the metal-SH complexes, saturation capability of MT with metal ions, colorimetric properties of reagents combined with mercaptans, immunoreactivity with specific antibodies, or metal moieties (Dabrio et al., 2002).

The electrochemical method based on Brdicka's reaction allows for determining total concentrations of MT, including metal-stripped and metal-binding MT (Brdicka, 1933; Pavicic et al., 1993; Raspor and Pavicic, 1996; Petrlova et al., 2007). However, MT concentrations obtained by this method might be overestimated because of the lack of a distinction between MT and other SH-containing proteins, so that careful pretreatment is required (Raspor and Pavicic, 1996). Compared to other methods, the spectrophotometric method is able to detect MT at low concentrations and facilitates simple, repeatable, and low-cost determination of MT (Viarengo et al., 1997). The chromatographic method allows to distinguishing different isoforms of MT while some disadvantages have been summarised, e.g., potential dissociation of metal-ligand complexes, interactions of metal ion, or metal-ligand complexes with column walls (Winge and Brouwer, 1986; Micallef et al., 1992; Ghazi et al., 2003). Immunological methods are the most sensitive, but systematic errors may occur due to the low specificity of the antibody (Dabrio et al., 2002).

The use of different methods for isolation and quantification of MT might lead to different results on MT induction (e.g., Zorita et al., 2005). A quality control of the MT determination is in most