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Metallothionein concentration in sponges (*Spongia officinalis*) as a biomarker of metal contamination

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Abstract

The synthesis of metallothioneins (MTs) is often induced when organisms are exposed to heavy metals in the field. They are among the major "specific" biomarkers identified to date. With a view to include MTs in biomonitoring programs, the organisms most commonly studied are bivalves. Sponges present most of the characteristics researched in bioindicators of pollution and consequently have been proposed to constitute a "Sponge Watch Program". The detection of large quantities of metals in sponges suggests the existence of detoxification systems and indeed, the presence of metallothionein-like proteins (MTLPs) has been reported in two different species of sponges. In *Spongia officinalis*, the present study has demonstrated the presence of compounds exhibiting most of the characteristics of MTs: cytosolic, heat-stable, with apparent molecular mass of 4 to 15 kDa and binding (at least) Ag, Cu and Zn. Specimens have been collected along the French Mediterranean coast from three sites differing by their degree of contamination. Relationships between MTLP and metal concentrations have been established. For copper, mercury and zinc, the correlations were significantly positive.

Keywords: Sponges; Cadmium; Copper; Mercury; Silver; Zinc; Metallothionein

1. Introduction

Among biomarkers which are currently used to monitor the quality of the marine environment, the induction of metallothioneins (MTs) has been proposed since this response to metal exposure is well documented (see reviews by Roesijadi, 1992; George and Olsson, 1994; Cosson and Amiard, 2000). In invertebrates, tolerance to metals is mainly based on sequestration by a large range of cellular ligands such as MTs, lysosomes, mineralized or organic-based concretions (Langston et al., 1998; Marigomez et al., 2002; Wallace et al., 2003), contrary to vertebrates in which metal-binding to MTs is considered to be the major route for detoxification. Thus George and Olsson (1994) have suggested that fish species would be better candidates for monitoring use based on the determination of MTs than invertebrates. However, due to the representativeness of sedentary invertebrates with regard to the local situation, the possible use of invertebrate MTs as a biomarker has given rise to a large number of studies which have been recently reviewed (Langston et al., 1998; Cosson, 2000; Isani et al., 2000). Mussels which have been widely used for chemical biomonitoring in Mussel Watch Programs (NAS, 1980; RNO, 2000) have been validated as a good biological matrix for the determination of MTs as a biomarker of response to metal contamination (Mourgaud et al., 2002 and literature cited therein).

Sponges have also most of the characteristics of good biomonitors as summarized by the US NAS (1980) and

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have been proposed for Sponge Watch Programs (Patel et al., 1985; Hansen et al., 1995). As filter-feeders, they accumulate in their tissues a number of chemicals (hydrocarbons, organochlorinated compounds, metals) present in the water column both in the soluble and particulate phases (Hansen et al., 1995; Cebrian et al., 2003; Pérez et al., 2003 and literature cited therein). Moreover, experimental studies have shown that accumulation of metals in sponges paralleled the concentrations of these contaminants in their medium (Hansen et al., 1995; Cebrian et al., 2003). The possible use of *Spongia officinalis* as an indicator of metal contamination has been assessed: twelve metals were surveyed at 9 sites, generating a pollution gradient around the conurbation of Marseille, France, and temporal trends were also examined considering samples collected before and 12 years after bringing into service a sewage treatment plant (Pérez et al., 2005). The overall contamination level registered provided a classification of the study sites which is congruent with that given by other studies on pollutant accumulation in neighbouring sandy-bottom or benthic assemblages (e.g. Bellan et al., 1999; Muricy, 1991). The significant decrease of metal concentrations (except for Ni) between 1984 and 1999 was consistent with an improvement of water quality due to the treatment of the effluents of Marseille. Thus this species seems a reliable indicator of metal bioavailabilities.

Metallothioneins or at least metallothionein-like proteins (MTLPs) are generally recognised to be ubiquitous in living beings (Binz and Kägi, 1999; Cosson and Amiard, 2000). In the marine sponge *Microciona prolifera*, Philp (1999) has described the presence of MTLP but had not plotted MTLP levels against metal contents. In *Suberites domuncula*, Schröder et al. (2000) have carried out gene sequencing and have shown that MTLP contained about 32% of cysteine but neither histidine nor aromatic amino-acids. These authors consider that the MTLP of *Suberites domuncula* exhibits most of the characteristics of class 1-MT.

The objectives of the present work were to verify the existence of MTLP in *Spongia officinalis* and to compare MTLP induction in specimens originating from metal-rich and comparatively clean sites chosen from among those previously investigated by Pérez et al. (2005). The ultimate aim is to propose a bioindicator of metal pollution for Mediterranean rocky communities complementary to mussels.

2. Materials and methods

2.1. Sampling

Sponges were collected from three sites along the French Mediterranean coast: Cortiou is located 300 m east of the discharge outlet of the sewage treatment plant of the conurbation of Marseille (1.5 million inhabitants, daily output of about 250,000 m^3). Niolon is located a few meters from an outlet responsible for a small input of domestic effluents (40 to 110 m^3 per day). Port-Cros is a control site, located in a National Park, 30 km off the coast, and 100 km east of Marseille.

The specimens were sampled at a depth about 10 m, on vertical rock-faces or on the ceiling of small caves where they are protected from sedimentation. Sponges of similar size were selected to avoid as far as possible variations of metal concentrations due to differences in age. Pieces were cut, kept in an isothermic container with seawater during the transport to the laboratory, then drained on absorbent paper, cut into pieces of about 2 g, then stored at −80 °C.

2.2. Characterization of metal-binding compounds and MTLP determination

In order to characterize metal-binding compounds, sponge tissues were homogenized by hand with agate mortar and pestle in liquid nitrogen, allowing disruption of the dense net of spongine fibres. Then homogenization was completed in 20 mM TRIS, 150 mM NaCl solution adjusted to pH = 8.6 (4 mL/g soft tissue). β-Mercaptoethanol (10 mM) was added to limit oxidation as well as phenylmethanesulfonyl fluoride (PMSF) as an anti-protease (0.1 mM). Soluble (S1) and insoluble (P1) fractions were separated by centrifugation (25,000 $\times g$ for 55 min at 4 °C). The cytosolic heat-stable thiolic compounds including MTLPs (S2) were isolated by centrifugation of the soluble fraction (15,000 $\times g$ for 10 min at 4 °C) after heat treatment (70 °C for 15 min) (Fig. 1).

The cytosol before (S1) or after (S2) heat-denaturation was fractionated by gel chromatography using a Sepharose CL 6B (Pharmacia) column (870 × 16 mm) or a Sephadex G75SF (Pharmacia) column (700 × 16 mm) equilibrated with the buffer used for elution (20 mM TRIS, 150 mM NaCl, pH = 8.6). Two replicates of S1 or S2 were used for each type of chromatography. An aliquot of S1 or S2 was applied to the column, eluted with buffer (48 mL h^{-1} for CL6B, 18 mL h^{-1} for G75SF) and collected as 2.4 mL fractions. The Sepharose CL6B column was calibrated for molecular mass estimations using standard markers (ribonuclease, chymotrypsinogen, thyroglobulin, ferritin). The Sephadex G75SF column was calibrated using ribonuclease, chymotrypsinogen, ovalbumin, and bovine serum albumin. The molecular mass of the different metal-binding compounds was derived from the calibration curve.

The amount of MTLPs was determined by differential pulse polarography, a technique based on-SH compound determination according to the Brdicka reaction (Brdicka, 1933) as described by Geffard et al. (2002) according to Thompson and Cosson (1984). This method has been validated in the framework of BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes,