A COMPARATIVE STUDY ON THE METALLOTHIONEIN CONTENT OF SIX MARINE BENTHIC ORGANISMS

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Abstract

The levels of the metallothionein content of five bivalves and one ascidian collected from two different sites ion the central Greek coast were determined spectrophotometrically using Ellman's reagent. The marine organisms used in this study can be found in quite diverse environments. The studied specimens were collected from the same areas and had been exposed to the same pollution levels. The results of this preliminary study showed that all investigated bivalves expressed very similar concentrations of metallothioneins. The ascidian showed lower levels of metallothioneins but still within the same order of magnitude.

Key-words: bivalves, metals, Aegean Sea

Introduction

The last decades great scientific efforts have been devoted towards the assessment of the bioavailability of metals in the aquatic ecosystems and the development of methods for the determination of their environmental impact [1,2].

Studies have shown that water and sediment analyses cannot be used to predict or assess environmental impact. It is known that benthic invertebrates are capable of accumulating metals at concentrations many thousand times greater than those present in the surrounding water and therefore can be used for determinations of the accumulation of metals in animals from polluted sites. Based on these facts the concept of "indicator organisms" for filter feeding bivalves, on suspended particulate metals was formulated [3, 4, 5, 6].

Metallothioneins are metal complexes of the sulfur rich protein, thionein and can be found in a wide array of marine organisms. Metallothioneins are complexes of small proteins with a molecular size ranging between 6.000 and 7.000 Daltons. MTs consist of 60-62 amino acids and contain 20 cysteine residues which do not form disulfide bridges. So far, four different charge isoforms of MTs have been identified and it has been suggested that the role of MT is to protect the organisms against the toxic effects of certain metals. The induction of MT synthesis was first demonstrated with high doses of cadmium and it was shown that once the synthesis of MTs is initiated, the producer organism can withstand higher levels of Cd^{2+}. Most of the studies on MT have been done in relation to cadmium, since it is one of the most toxic metals. Other essential trace metals such as Cu and Zn were proven capable to complex with MTs as well.

The major consideration towards the "bioindicators" approach, for the assessment of environmental impact, is the selection of the most suitable organism that could be used globally for the "biomonitoring". Several marine invertebrates, including commonly occurring species such as worms, molluscs (including clams, mussels, oysters and scallops) and crustaceans possess specialized mechanisms for immobilizing and accumulating excess metals in specific organs, cell types or in specific metallothioneins [7, 8, 9, 10].

Extensive work has been done on the metal uptake by oysters (*Crassostrea* or *Ostrea* spp) for the reason that oysters rather than mussels are found in most areas of the eastern coast of the USA. It is known that oysters possess a metallothionein of 75 amino acids with 21 cysteine residues the synthesis of which is induced upon exposure of the oyster to cadmium. The difficulty with these organisms is the occurrence of naturally high metal levels, metal displacement reactions and competition between sequestration mechanisms [11].

One of the organisms that combines the majority of the selection criteria is *Mytilus galloprovincialis* (Lamarck). This mussel species is quite common in most of the Mediterranean coastal areas. Even though *M. galloprovincialis* is a common bivalve in the Mediterranean basin there are still many areas that it cannot be found. The habitat of the *M. galloprovicialis* is the littoral and sublittoral zone and is very infrequently found at depths greater than 5 meters. The scope of this study is to determine the MT levels in some of the common bivalves and mussels found around the coasts of Greece and correlate those with the values obtained from the respective assays employing *M. galloprovincialis*.

Methods and materials

In order to find organisms that could provide supplementary data to those obtained from *M. galloprovincialis*, in the framework of an environmental assessment programme, five of the most common marine benthic organisms were investigated. The other studied organisms were: *Chlamys varia* (Linnaeus), *Cerastoderma edule* (Linnaeus), *Venus verrucosa* (Linnaeus), *Mactra corallina* (Linnaeus) and *Phallusia mammilata* (Cuvier). These organisms can be found in a wide array of habitats and depths (2-140 meters). Some of them grow on rock formations and others are burrowing in sand, gravel or mud. *C. edule* can tolerate salinities between 3,4% and 4,0% [12]. The specimens used in our studies were hand collected by scuba divers and were brought alive to the laboratory for the determination of MTs. The collection sites were the Gulf of Elefsis (Neraki) and the Gulf of Chalkis (Chalia). Care was taken to use individuals of the same size, since size roughly represents the age of the organisms. The specimens were found no more than 100 meters apart from each other. All species were identically treated in order to minimize experimental errors.

The bivalves were dissected and the digestive glands were removed and homogenized. In the case of *Phallusia mammilata* only part of the digestive track of the ascidian was used for the assays. The experimental protocol applied was the spectrophotometric assay suggested by Prof Viarego [1] for the determination of MTs in *M. galloprovincialis*. The small size proteins were isolated from the homogenized tissue, through a series of precipitations and resolubilizatios and were allowed to react with DTNB (5,5-dithiobis-2-nitrobenzoic acid). The colored solutions were then analyzed photometrically and based on the values of standard solutions; the absorbancies were correlated to concentrations of MTs (μg of MT/gr of tissue).

Results and discussion

Between 4 and 7 samples from every organism were assayed and the average of the values along with the standard deviation is shown in the following table.

Table 1: Concentration of metalothioneins (μg/ g of tissue) in selected organisms

organism	Average Concentration	Standard Deviation
Mytilus galloprovincialis	175.869	27.435
Chlamys varia	264.041	38.765
Cerastoderma edule	198.207	4.828
Venus verrucosa	172.401	13.854
Mactra corallina	240.484	20.553
Phallusia mammilata	101.670	12.263

It is clear from the above data that the concentrations of MTs in all investigated organisms are comparable. *C. varia* and *M. corallina* exhibited the highest and *P. mammilata* the lowest levels of MTs. It is worth noting that *C. edule*, among all organisms investigated showed the smallest individual variation in the concentration of MTs.

In order to confirm that the highest levels of MTs are found in the digestive track of the organisms, the gills and the digestive gland from a set of *C. edule* were dissected and separately analyzed (Table 2).

Table 2: Comparison of the metallothionein levels in different tissue types of the same organism.

Tissue	Average Concentration	Standard Deviation
Digestive gland	198.207	4.828
Gills	120.394	5.053