BIOCHEMICAL BIOMARKERS RESPONSES TO SUSPENDED AND RE-SUSPENDED PARTICLES *IN SITU*

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Abstract

Three biomarkers [acetylcholinesterase (AChE), glutathione *S*-transferase (GST) and metallothioneins (MTs)] were measured in *Mytilus galloprovincialis* exposed to suspended and re-suspended particles *in situ*. Results provided not only temporal evidence that contaminants have been sorbed in suspended and re-suspended particles which were bioavailable, but also indicated significant difference of biomarkers responses between suspended and re-suspended particles.

Key-words: biomarker, AChE, GST, metallothionein, resuspension

Introduction

Contaminants that are present in marine environment are typically free or bound on suspended particles and sedimentary materials where they represent a source of chronic contamination and threat to pelagic and benthic organisms. Sedimentary material may periodically subjected to re-suspension processes resulting from natural events such as storm occurrences or bioturbation and human activities like dredging, shipping or trawling [1, 2]. The Mediterranean coastal zones experience such events either by near-bed current activity and breaking of internal waters or intense trawling operations resulting to significant re-suspension release of nutrients, pollutants or other toxic elements [3, 4]. Although these processes have been identified as being significant only little work has been devoted to their effects on biota [5]. The last decade biological effects are detected with tools that can provide early warning signals caused by the wide variety of contaminants present in the marine environment. Such tools are called biomarkers and are indicative of contaminant exposure and/or effect. The importance of measuring several biomarkers at the same time in the same organism may enables not only a pertinent approach to evaluating the effects of contaminants on individuals and the bioavailability of toxicants, but also the source of the exposure. This study was undertaken as a step in using biochemical biomarkers to determine responses caused by contaminants sorbed in suspended and re-suspended particles *in situ*.

Material and methods

A mooring device of a mud-hook, an auto-releaser and two bunches of nets with 50 mussels (*Mytilus galloprovincialis*) each, was placed in Thermaikos Gulf (northeastern Aegean Sea) and was exposed to suspended and re-suspended matter for 20 days during September and February 2001. The months correspond to periods of low and high re-suspension events occurred in the area. Net bunches were connected and suspended on a rope. One net was suspended at 10 m depth from the surface while the second 5 m above the sea-bottom level (50m). After collection, mussels were transferred to the lab and were stored at –80ºC till analysis. Three biochemical biomarkers were measured: acetylcholinesterase (AChE), glutathione *S*-transferase (GST) activities and metallothioneins (MTs) concentration. AChE as indicative for organophosphorous and carbamates since it is inhibited by their presence [6]. GST as indicative for organic compounds like organochlorine pesticides and PCBs as inhibited in their presence [7] and MTs concentration which increase in the presence of heavy metals. Determinations were carried out as have been previously described. AChE and GST were measured in the gills in the post-mitochondrial fraction (S9). AChE was measured according to [8] as modified by [9]. GST activity was determined according to [10]. MTs concentrations were determined in the digestive gland of mussels according to [11] and expressed as μg of MTs per gram tissue. AChE and GST activities were expressed as nmoles/min/mg of S9 protein (mg P). Protein concentration was determined according to [12].

Results and discussion

Results (average and standard deviation) of biomarkers responses measured in the gills and digestive gland of mussels are shown in Table 1. During the two periods (September, February), AChE activities and MTs concentration values for samples placed near to the surface level were slight decreased, and GST activities for similar samples were increased. These results were mainly related to the seasonal variation and to the different life cycle of the mussels which have also been reported by others [7, 9, 11]. It is however notable for samples placed near to the bottom level during February since biomarkers responses were significantly decreased compared to all the other samples. Decreases of approximately 37%, 25% and 7% were indicated for AChE, GST and MTs, respectively. It is clear from the data shown that biomarker responses provided not only temporal evidence that contaminants have been sorbed in suspended and re-suspended particles and were bioavailable but also indicated the source of the exposure (i.e. suspended and/or re-suspended particles).

Table 1. Biochemical biomarker responses of *Mytilus galloprovincialis* exposed to suspended and re-suspended particles *in situ*.

Source of exposure	Period of exposure	AChE (nmoles/min/ mg P)	GST (nmoles/min/ mg P)	MTs (_g/g tissue wt)
1. Suspended particles	Sept.r	52.77 ± 9.28	157.99 ± 8.31	81.16 ± 2.67
2. Re-suspended particles		52.98 ± 13.56	84.81 ± 11.56	80.17 ± 2.17
3. Suspended particles	February	42.50 ± 10.59	226.14 ± 26.29(2)	61.24 ± 1.17 (1,2,4)
4. Re-suspended particles		26.72 ± 12.71(1,2,3)	168.96 ± 23.99(2)	56.97 ± 0.99(1,2,3)

* Number in parenthesis indicate significant differences at $P<0.05$ level.

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