Bull. Environ. Contam. Toxicol. (1994) 53:726–732

Environmental Contamination and Toxicology

Involvement of Metallothionein in Cadmium Accumulation and Elimination in the Clam *Ruditapes Decussata*

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Received: 12 August 1993/Accepted: 28 March 1994

Cadmium is one of the most toxic pollutants in seawater because of its persistence, toxicity and potential for bioaccumulation. It is included on the "black list" of several international agreements established to regulate the input of pollutants into the marine environment (Yeasts and Bewers 1987).

The deleterious effects of cadmium contamination in marine organisms result from its accumulation within specific tissues. However, most of these organisms have developed subcellular detoxification processes, including the synthesis of metallothioneins, low-molecular weight, metal-binding proteins (Bebianno and Langston 1991, 1992; Bebianno *et al.* 1992; Roesijadi 1992; Walkes and Goering 1992).

Bivalves have the ability to accumulate and concentrate cadmium to levels several orders of magnitude above those found in their environment. The present study was designed to examine the involvement of metallothionein synthesis in cadmium accumulation and elimination in the bivalve *Ruditapes decussata* when exposed to a sublethal cadmium concentration (100 µg/l) and to a mixture of cadmium (100 µg/l), copper (50 µg/l) and zinc (50 µg/l).

MATERIALS AND METHODS

Ruditapes decussata (shell length 32±3 mm, mean dry weight 0.30±0.07 g) were collected from the Ria Formosa lagoon (South of Portugal) and acclimated in aerated seawater, salinity 36±1‰ and 20±2°C, for one week prior to the experiments. Sixty clams were held in 5 L and exposed to a cadmium concentration of 100 µg l^{-1} for up to 40 days, after which they were transferred to clean seawater for fifty days. A further group of 60 clams was maintained in

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clean seawater throughout the experiment as controls. Water in each tank was changed twice a week. Samples of six control and six treated clams were removed for analysis of cadmium concentrations after 7, 14, 21, 30 and 40 days of exposure and after 20 and 50 days in clean seawater. In addition, metallothionein concentrations were determined in controls, in clams treated for 40 days, and in clams detoxified for 50 days in clean seawater.

In a separate experiment, 60 clams were exposed to a mixture of cadmium (100 µg/l), copper (50 µg/l) and zinc (50 µg/l). A sample of six clams was taken for cadmium analysis after 7, 14, 21 and 30 days of exposure.

The soft parts of *Ruditapes decussata* were removed from the shell. The gills, digestive gland and remaining tissues were dissected into individual tissues. Six samples of each individual tissue were pooled, weighed and dried for subsequent cadmium analysis. The tissues of a further six individuals were pooled and homogenized in three volumes of 0.02 M Tris-HCl (pH 8.6) buffer in an ice-bath. Subsamples were taken for determination of wet/dry weight ratio. Aliquots of the homogenate (3 to 5 ml) were centrifuged at 30,000 ***g*** for 1 hr at 4°C. The supernatant (cytosol) was separated from the pellet, heated at 80°C for 10 min, to precipitate the high molecular weight (HMW) proteins, and subsequently centrifuged at 30,000 ***g*** for 1 hr at 4°C. Aliquots (50 to 250 µl) of the heated cytosol were taken for quantification of metallothioneins (MT) by differential pulse polarography, as described in Bebianno and Langston (1989), using a polarograph Methrom 646. Metallothionein concentrations were determined as mg g^{-1} dry weight of homogenized tissue.

Cadmium was analyzed in dried, HNO_3-digested subsamples of the gills, digestive gland and remaining tissues using flame or furnace atomic absorption spectrophotometry. Analysis of Cd in TORT I lobster hepatopancreas (National Research Council Canada) resulted in values of 26.98±0.01 µg g^{-1} compared with a certified value of 26.3±2.1 µg g^{-1}. All metal concentrations were expressed on a dry tissue weight basis. All data were analyzed statistically using ANOVA with a significance level of 0.05.

RESULTS AND DISCUSSION

Accumulation of cadmium and subsequent depuration in the gills, digestive gland and remaining tissues of treated clams are presented in Fig. 1.

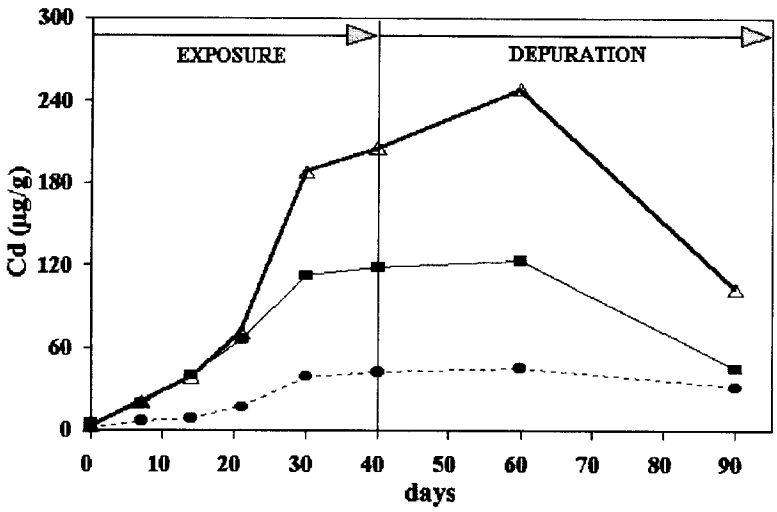

Figure 1. Total cadmium concentrations in the gills (■), digestive gland (Δ) and remaining tissues (•) of *Ruditapes decussata* exposed to cadmium (100 $\mu g\ l^{-1}$) for 40 days and detoxified for 50 days. Samples pooled from six individuals.

Concentrations of cadmium in tissues of controls did not change during the course of the experiments, with the highest concentrations occurring in the digestive gland (6.1±0.34 $\mu g\ g^{-1}$) followed by the gills (4.8±0.99 $\mu g\ g^{-1}$) and the remaining tissues (2.1±0.34 $\mu g\ g^{-1}$). In comparison with the controls, cadmium increased in all the tissues of the treated clams during the initial 30 days of exposure ($P<0.05$). Cadmium accumulation rate was highest in the digestive gland (5.8 $\mu g\ g^{-1}\ d^{-1}$, $P<0.01$), followed by the gills (3.6 $\mu g\ g^{-1}\ d^{-1}$, $P<0.001$) and the remaining tissues (1.2 $\mu g\ g^{-1}\ d^{-1}$, $P<0.01$). The concentration of cadmium in the digestive gland exceeded that in the gills but only after 14 days of exposure (Fig. 1). This suggests that cadmium in the gills is transported to the digestive gland for storage, which concurs with earlier observations for the same species (Bebianno *et al.* 1993), for the mussels *Mytilus edulis* (Scholz 1980) and *Mytilus galloprovincialis* (Viarengo *et al.* 1985), and for the gastropod *Littorina littorea* (Bebianno *et al.* 1992).

After 30 days of exposure to cadmium the accumulation was much slower indicating the approach of equilibrium (Fig. 1). At the end of the exposure period, the concentrations of cadmium in the digestive gland, gills and remaining tissues at treated clams were, respectively, 34, 25 and 20 times higher than those in the tissue of control clams. Similar patterns of cadmium accumulation have been observed for the clam *Ruditapes decussata,* for the mussels *Mytilus edulis* and *Mytilus galloprovincialis,* and for the gastropod *Littorina littorea* exposed to 400µg Cd l^{-1} over the same period (Bebianno & Langston 1991, 1992; Bebianno *et al.* 1992; Bebianno *et al.* 1993).

Table 1. Cadmium concentrations in tissues of *Ruditapes decussata* exposed to 100 µg Cd l^{-1} and to a mixture of 100 µg Cd, 50 µg Cu and 50 µg Zn/l after 30 days of exposure.

Tissue	Control µg/g	Cd µg/g	Cd+Cu+Zn µg/g
Whole soft tissues	3.6	85.4	88.6
Gills	5.1	112.5	131.3
Digestive gland	6.4	188.6	195.8
Remaining tissues	2.2	39.1	36.7

The accumulation of cadmium in the tissues of *R. decussata* exposed for 30 days to cadmium mixed with copper and zinc is compared in Table 1 with the accumulation of cadmium in clams exposed to only cadmium over the same period. In comparison with the controls, the pattern of cadmium uptake was similar, although the final concentrations of cadmium in the tissues of clams exposed to the mixture of metals were higher except in the remaining tissues than those treated only with cadmium. It is possible that cadmium might be replacing zinc and copper in the tissues (unpublished data). Vicente *et al.* (1988) carried out a similar experiment with this species of clam at 14°C and similar concentrations of cadmium. They observed lower accumulation levels of cadmium than those obtained in the current experiment which can be attributed, perhaps, to the lower temperature. Henry *et al.* (1982) also noted a direct correlation between cadmium uptake and temperature in this species.

When the clams were transferred from treated seawater, the levels of cadmium continued to increase in all tissues, particularly in the digestive gland, for up to 20 days in clean seawater, before starting to decline (Fig. 1). Within 50 days of depuration, the concentration of cadmium in the gills, digestive gland and remaining tissues declined by 62%, 51% and 26%, respectively. Even at the end of the depuration period, these tissue still retained 9, 29 and 17 times more cadmium, respectively, than did the equivalent tissues from the controls. These results show that the loss of cadmium is a slow process and that the rate of release depends on the tissues involved, possibly reflecting different routes of cadmium metabolism. The half-life for cadmium in these tissues was not calculated because of the lack of data during the period of depuration. In the case of the oyster *Crassostrea virginica* and the gastropod *Littorina littorea* it was suggested that cadmium was transferred from the gills to internal tissues during detoxification, since the turnover rate was much lower in the latter relative to the former tissues (Roesijadi and Klerks 1989; Langston and Zhou 1987). In *Mytilus galloprovincialis* previously exposed to 200 µg Cd/l for 28 days, there was a 50% decrease in cadmium levels within the gills and digestive gland after 4 months of detoxification (Viarengo *et al.* 1985).

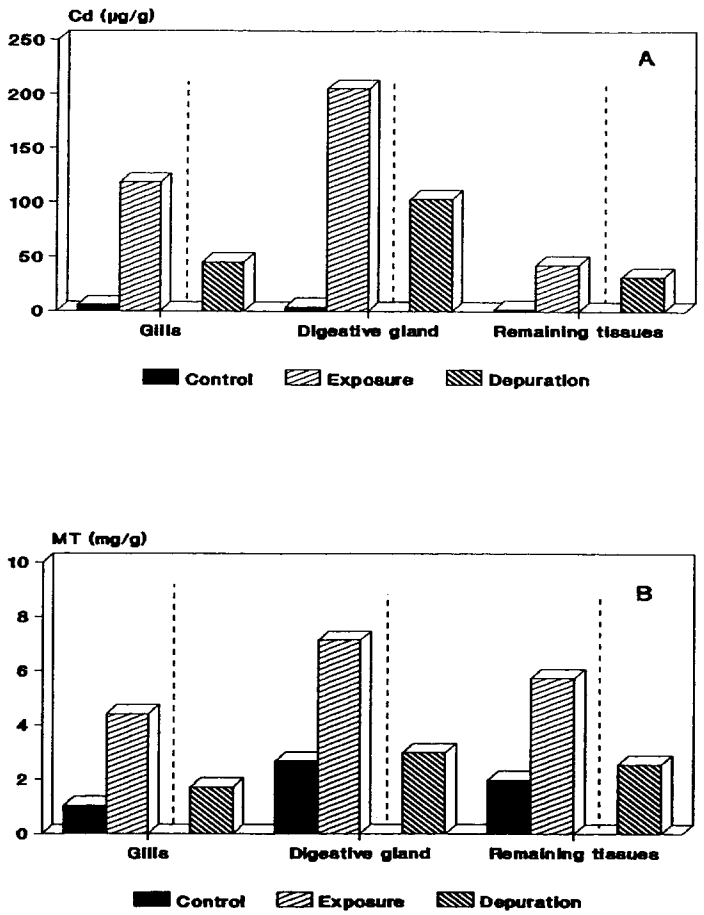

Figure 2. (A) Total cadmium concentrations and (B) metallothionein concentrations in the gills, digestive gland and remaining tissues of *Ruditapes decussata* controls and clams exposed to cadmium for 40 days and detoxified for 50 days. Samples pooled from six individuals.

R. decussata shows a capacity for accumulating and surviving high concentrations of cadmium, which suggests that at least some of its tissues are capable of synthesis of metallothioneins which inhibit the toxic effects of this pollutant (Bebianno *et al.* 1993). A comparison of the concentrations of cadmium and metallothionein in the gills, digestive gland and remaining tissues are presented in Fig. 2 for clams that were maintained as controls; exposed to cadmium for 40 days; and depurated for 50 days after exposure to a cadmium solution (100 µg l^{-1}) for 40 days. In control clams, there was a reduction in the concentration of metallothionein from the digestive gland (2.45±0.38 mg g^{-1}) to the remaining tissues (1.96±0.72 mg g^{-1}) and finally to the gills (1.03±0.22 mg g^{-1}). Clams exposed to cadmium for 40 days showed an increase in metallothionein levels

which was four-fold in the gills and three-fold in the other tissues (ANOVA, $P<0.05$) (Fig. 2). After depuration for 50 days, there was a decrease in the concentration of metallothionein to levels which were slightly higher than those estimated for the controls. In the gills, the difference was still two-fold but, for the other tissues, the difference was much lower, suggesting that the turnover of metallothionein, although different in the three tissues, was faster than cadmium turnover. The results provide evidence for the induction of metallothionein in the clams exposed to cadmium, confirming earlier work on this species (Bebianno *et al.* 1993) and on *Mytilus edulis* (Pavicic *et al.* 1992; Bebianno and Langston 1991), *M. galloprovincialis* (Bebianno and Langston 1992) and in *Littorina littorea* (Bebianno *et al.* 1992). The greater induction of metallothionein in the gills compared to the other tissues observed in *Littorina littorea* and *Crassostrea virginica* (Roesijadi and Klerks 1989) suggests that metallothioneins in these tissues are involved in the elimination of cadmium by the three tissues of the clams.

These results suggest that metallothioneins are involved in the accumulation and in the elimination of cadmium from the gills, digestive gland and remaining tissues. Consequently, estimation of metallothionein in the gills can be used as a sensitive measure of biological response of cadmium exposure.

Acknowledgements. M.J. Bebianno was supported by a research grant from the European Environmental Reseach Organization (EERO). The authors also thank Dr J. D. Icely for the English revision of this paper.

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