BioMetals (2006) 19:237–244
DOI 10.1007/s10534-005-5671-9

Cytochrome *c* oxydase subunit I gene is up-regulated by cadmium in freshwater and marine bivalves

Maud Achard-Joris, Patrice Gonzalez, Véronique Marie, Magalie Baudrimont & Jean-Paul Bourdineaud*
*Laboratoire d'Ecophysiologie et d'Ecotoxicologie des Systèmes Aquatiques (LEESA), Université Bordeaux1/ UMR CNRS 5805, Place du Dr. Peyneau, 33120, Arcachon, France; *Author for correspondence (Tel: +33-556-22-39-26; Fax: +33-556-54-93-83; E-mail: jp.bourdineaud@epoc.u-bordeaux1.fr)*

Received 28 January 2005; accepted 18 April 2005

Key words: biomarker, bivalves, cadmium, *CoxI* gene, quantitative real-time PCR, zinc

Abstract

Inhibition of the mitochondrial electron transfer chain and induction of reactive oxygen species (ROS) production are one of the roots of cadmium (Cd) toxicity. To appreciate the impact of Cd on mitochondria, we focused on the expression of *CoxI* gene which encodes the subunit I of the Cytochrome *c* oxidase (complex IV of the respiratory chain). *CoxI* gene expression was studied by real-time quantitative PCR in three species: two freshwater bivalves (*Corbicula fluminea* and *Dreissena polymorpha*) and one marine bivalve (diploid or triploid *Crassostrea gigas*). Bivalves were exposed for 10 or 14 days to 0.13 μM Cd^{2+} and 15.3 μM Zn^{2+} in controlled laboratory conditions. We demonstrate that in the three mollusk species *CoxI* gene was up-regulated by Cd. Zinc (Zn), which is known to have antioxidant properties, had no effect on *CoxI* gene expression. In the presence of Cd and Zn, *CoxI* gene inducibility was lower than after a single Cd exposure, in each species; result that could not be fully explained by a decreased Cd accumulation. *CoxI* gene induction by Cd was 4.8-fold higher in triploid oysters than in diploid ones, indicating a possible influence of triploidy on animal responses to Cd contamination.

Introduction

Heavy metals, depending on their oxidation states, can be highly reactive and hence toxic to most organisms. Metals such as copper (Cu) and zinc (Zn) are essential for life, whereas others, such as mercury (Hg) and cadmium (Cd), are not known to perform any useful biochemical function. Cd in particular is very toxic and is considered a serious occupational and environmental health threat since it is a metal with many industrial uses which is continuously introduced into the atmosphere, into aquatic and terrestrial environments. Toxicological responses of Cd exposure include kidney damage, respiratory diseases, neurological disorders and several kinds of cancer (Waalkes *et al.* 1992).

To counter Cd toxicity, living organisms use several metal resistance mechanisms. Cells respond by increasing the expression of genes encoding a variety of proteins (Carginale *et al.* 2002): toxic ions can be segregated by thiol-containing molecules like metallothioneins (Roesijadi *et al.* 1996) or pumped out of the cell via a membrane protein (Li *et al.* 1997; Achard-Joris *et al.* 2005). A variety of mechanisms have been attributed to Cd toxicity. It interferes with the intracellular signaling network and gene regulation at multiple levels (Beyersmann & Hechtenberg 1997) and may be associated with the production of reactive oxygen species (ROS, molecules and free radicals such as superoxide anion and hydrogen peroxide derived from molecular oxygen) (Szuster-Ciesielska *et al.* 2000). Under normal conditions the production

and destruction of ROS is well regulated in cell metabolism, but under environmental oxidative stress the balance between prooxidative and antioxydative reactions is shifted in favor of the former.

As mitochondria are a major source of superoxide and hydrogen peroxide production in cells (Turrens 1997) several studies have focused on the effect of Cd on mitochondrial metabolism: Cd has been shown to inhibit the mitochondrial electron transfer chain and to induce ROS production (Wang *et al*. 2004). Moreover, Cd-promoted oxidative stress leads to DNA strand damage – especially mitochondrial ones (Yakes & Van Houten 1997) – and to apoptotic cell death (Bagchi *et al*. 2000). This suggests that enhanced ROS production may be at the root of Cd toxicity. Two more ways in which cells might avoid Cd toxicity can then be proposed: hypertrophied antioxidant defenses or increased consumption of molecular oxygen. As Cd can induce alterations in the activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase after only a few hours exposure (Company *et al*. 2004; Ikediobi *et al*. 2004), we focused on the second way and especially on Complex IV (Cytochrome *c* oxidase (COX)) of the mitochondrial respiratory chain. This transfers electrons from reduced Cytochrome *c* to molecular oxygen, and contributes to the production of an electrochemical proton gradient across the mitochondrial inner membrane that drives the synthesis of ATP (Capaldi *et al*. 1983; Capaldi 1990). Complex IV is particularly interesting because it has been shown that oxygen-tolerant strain of HeLa cells had twice the Cytochrome *c* oxydase activity of the wild-type ones, and inhibition of Cytochrome *c* oxydase enhanced ROS production (Campian *et al*. 2004). Assuming that cell damage does arise from exaggerated mitochondrial ROS production, the site of electron leak from the electron transport chain becomes an important question.

To study the impact of Cd toxicity on mitochondria we looked at *Cox* subunit I mitochondrial gene (*CoxI*) expression. An advantage of using COX to study the impact of Cd on mitochondrial metabolism is that *CoxI* gene sequence is highly conserved between lower and higher eukaryotes (Capaldi 1990). Thus a similar *CoxI* gene regulation between species can be expected.

Many aquatic organisms are of potential interest as ecologically sensitive targets of metallic pollution. Among them, bivalve mollusks are regularly used as bioindicators of water metal pollution because of their high bioaccumulation capacities for heavy metals (Inza *et al*. 1997) and because they are well represented in aquatic ecosystems. In this study, three aquatic species were chosen: two freshwater bivalves (the Asiatic clam *Corbicula fluminea* and the zebra mussel *Dreissena polymorpha*) and one seawater bivalve (the Pacific oyster *Crassostrea gigas*). Triploid *C. gigas*, oysters developed to enhance the production and selling period of commercial bivalves, were also used to investigate whether a third set of chromosomes could influence *CoxI* gene expression after metal contamination. *CoxI* gene expression level was studied in gill tissues because gills are the organ at the interface with the polluted environment and are thus the main tissues from which the metal loading in the organism will proceed (Marigomez *et al*. 2002). Cd concentration was set to 0.13 μM which is close to that found in a Cd contaminated river used for field contaminations (Achard *et al*. 2004). Since Zn has antioxidant properties (Bray & Bettger 1990), bivalves were also contaminated with Zn and a mix of Cd and Zn.

Materials and methods

Animals

C. fluminea (2 ± 0.2 cm shell length) were collected from a reference site in Cazaux-Sanguinet freshwater lake (Aquitaine region, south-west France). *D. polymorpha* (2.7 ± 0.2 cm shell length) were collected in the Garonne river, close to Agen (Aquitaine region, France).

Diploid and triploid *C. gigas* oysters (12 months old, 5.7 ± 0.1 cm shell length) were obtained from an oyster farmer in Arcachon Bay (Aquitaine region, France).

Metal contaminations

For freshwater bivalves, laboratory experiments were carried out using experimental units (EUs) based on glass tanks lined with plastic bags containing pure sand and dechlorinated tap water

permanently aerated by a diffuser connected to an air pump (Achard *et al.* 2004). Groups of 20 *C. fluminea* and 20 *D. polymorpha* were exposed for 10 days to $ZnCl_2$ (15.3 μM Zn^{2+}), $CdCl_2$ (0.13 μM Cd^{2+}) and $ZnCl_2$ + $CdCl_2$ (15.3 μM Zn^{2+} and 0.13 μM Cd^{2+}). Metal concentrations were maintained constant during the whole experiment by daily additions of aqueous Cd and Zn solutions, adjusted according to the decrease in metal concentration in each EU (determined by atomic absorption spectrometry – VARIAN AA 400, Victoria, Australia). The bivalves were fed every two days with 3×10^5 green algae (*Scenedesmus subspicatus*) per ml culture.

Experiments on oysters were performed using EUs based on polyethylene tanks containing aerated (281 μM of dissolved O_2) artificial seawater prepared by mixing Instant Ocean synthetic sea salt (Mentor, OH, USA; 25 ± 0.5‰) with dechlorinated tap water. Groups of 36 *C. gigas* diploid and 36 *C. gigas* triploid were exposed for 14 days to $ZnCl_2$ (15.3 μM Zn^{2+}), $CdCl_2$ (0.13 μM Cd^{2+}) and $ZnCl_2$ + $CdCl_2$ (15.3 μM Zn^{2+} and 0.13 μM Cd^{2+}). The contaminated water was changed daily to maintain Zn and/ or Cd concentrations constant. Oysters were contaminated 4 days longer than freshwater bivalves in order to obtain an equivalent Cd concentration in tissues. Indeed, for a given metal concentration, levels of free metal ions are much less in seawater than in tap water due to the presence of metal chloride species which are less available (Campbell 1995). Oysters were fed every two days with 0.05 g green algae (*Isochrysis galbana*) per oyster.

All the experiments were performed at 20 ± 0.3 °C, pH 8.5 ± 0.3 and with 12 h light per day according to a protocol already described (Achard *et al.* 2004). Cadmium chloride ($CdCl_2$) and zinc chloride ($ZnCl_2$) were purchased from Merck.

Cadmium and zinc determinations

The whole soft body of one bivalve was taken from each of the three replicates for each condition, then dissected, dried on absorbent paper and weighed (fresh weight, f.w.). Tissues and water samples were then analyzed for metal bioaccumulation by atomic absorption spectrophotometry as previously described (Achard *et al.* 2004).

Inter-individual variability for each experimental condition was defined by mean ± standard error (SE). Significant differences between metal concentrations in the whole soft body were determined using the nonparametric Mann–Whitney *U*-test ($P < 0.05$).

cDNA fragment production and SYBR green real-time PCR

Total RNA were extracted from gills of bivalves (two animals per reaction) using the Absolutely RNA RT-PCR Miniprep kit (Stratagene) according to the manufacturer's instructions. Three microgram of RNAs from laboratory bivalves were retrotranscribed in 50 μl reaction using the ProStar First-Strand RT-PCR kit (Stratagene).

Primers COXI5 5′-GGAATACCACGACG GTACTCT-3′ and COXI3 5′-AGGGCAGCC GTGTAAT-3′ were used on cDNA prepared from *D. polymorpha* and *C. gigas* RNA and primers CFCOXI5 5′-GATGGGTGGTTTTGGAAATT GAC-3′ and CFCOXI3 5′-ACTGACGGGCCA GAATG-3′on cDNA prepared from *C. fluminea* RNA. *CoxI* gene sequence from these three species were available in GenBank (*D. polymorpha*: AF120663; *C. gigas*: AF280608; *C. fluminea*: AF120666) and primers were designed according to the LightCycler probe design software (version 1.0, Roche). Normalization was carried out detecting cDNA 28S with the following primers: 5′-CCA AGG AGT CTA ACA TGT GC-3′ and 5′-GAT GGT TCG ATT AGT CTT TC-3′. Quantitative real-time PCR assay was carried out in 20 μl PCR mixture volume consisting of 1 μl LightCycler FastStart reaction mix SYBR Green I (containing FastStart *Taq* DNA polymerase, 0.3 μM of each oligonucleotide; Roche), 5 mM $MgCl_2$, 0.3 μM of each primer and 3 μl of cDNA. Gene amplification was carried out as follows: initial activation of HotStart Taq DNA Polymerase at 95 °C for 10 min; 50 cycles in three steps: 95 °C for 5 s, 60 °C for 5 s, 72 °C for 20 s. At the end of amplification cycles, melting temperature analysis was carried out by slow increase in temperature (0.05 °C s^{-1}) from 65 up to 95 °C. Amplification, data acquisition and analysis were carried out with a LightCycler instrument (Roche, Mannheim, Germany) using LightCycler 5.3.2 software (Roche). Three replicates were performed, meaning three

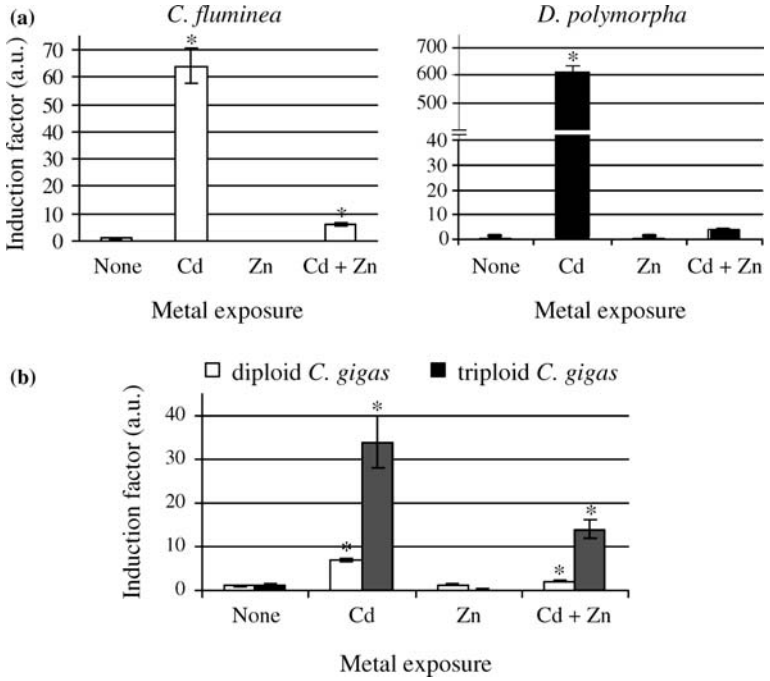

Figure 1. (a) Expression levels (±SE) after normalization for *CoxI* transcript in gills of *C. fluminea* and *D. polymorpha* exposed for 10 days to cadmium (0.13 μM Cd^{2+}), zinc (15.3 μM Zn^{2+}), or cadmium and zinc (0.13 μM Cd^{2+} and 15.3 μM Zn^{2+}). (b) Expression levels (±SE) after normalization for *CoxI* transcript in gills of diploid and triploid *C. gigas* exposed for 14 days to cadmium, zinc, or both metals at the same concentrations as in a. Expression ratio between *CoxI* and 28S genes in control bivalves was set to one. a.u. stands for arbitrary units. Measurements were done in triplicate. *Statistically different compared to control ($P < 0.05$).

independent RNA extractions and retrotranscriptions for each contamination condition. Relative quantifications of *CoxI* gene expression levels were normalized according to the 28S rRNA gene expression. Expression ratio between *CoxI* and 28S genes in control bivalves was set to one. Inter-individual variability for each experimental condition was defined by mean ± SE ($n = 3$). Significant differences between *CoxI* gene expression levels in the gills were determined using the nonparametric Krustal–Wallis method ($P < 0.05$) for more than two samples comparisons. If the test was rejected, we performed the Mann–Whitney *U*-test ($P < 0.05$).

Results

Bivalves were exposed to Cd, Zn, or both metals in controlled conditions. After Cd exposure, no mortality was observed and bivalves accumulated Cd well. The Cd accumulation factor (defined as Cd concentration in Cd-contaminated bivalve versus Cd concentration in control bivalve) was 79 in *C. fluminea*, 56 in *D. polymorpha*, 15 in diploid*C. gigas*, and 16 in triploid*C. gigas*. Cd concentration in the whole soft body of bivalves was similar in each species (around 48 nmol Cd g^{-1} fw.) (Table 1). After Cd and Zn exposure, a difference in Cd accumulation was observed only in *D. polymorpha*: Cd concentration was 2.4 times lower in mussels exposed to Cd and Zn than in the one exposed to Cd (Table 1); the 2.2 times lower Cd concentration observed in *C. fluminea* was not statistically significant.

Zn concentration in the whole soft body of control bivalves was different for each species. Indeed, in *C. gigas*, it was 13 and 53 times higher than in *C. fluminea* and *D. polymorpha*, respectively (Table 2). Although Zn basal levels were different for each species, similar Zn accumulation factors (defined as Zn concentration in Zn-contaminated bivalve versus Zn concentration in control bivalve) were observed between diploid*C. gigas*, triploid*C. gigas*, and *C. fluminea* after Zn exposure. Zn accumulation factor in *D. polymorpha* was calculated to be 18 and was six times higher than in the other bivalves (Table 2).

Table 1. Cadmium concentrations (measured in nanomol per gram fresh weight ± SE) in the whole soft body of bivalves after exposure to Cd, Zn, or Cd and Zn.

Species	Metal exposure, nmol Cd g^{-1} (fw)			
	None	Cd	Zn	Cd + Zn
C. fluminea[a]	0.6 ± 0.1	47.3 ± 23.1[c]	0.9 ± 0.04	20.9 ± 10.4[c]
D. polymorpha[a]	1 ± 0.2	55.5 ± 7.4[c]	1.1 ± 0.2	23.6 ± 5.2[c]
C. gigas diploid[b]	3.4 ± 0.2	52.4 ± 9[c]	3.2 ± 0.4	40.7 ± 3.8[c]
C. gigas triploid[b]	2.6 ± 0.3	41.4 ± 7.5[c]	2.4 ± 0.1	61.2 ± 5.2[c]

[a]*C. fluminea* and *D. polymorpha* were contaminated for 10 days. [b]Diploid or triploid *C. gigas* were contaminated for 14 days. [c]Significantly different to control ($P < 0.05$). Cadmium determination was done in triplicate for each condition.

Table 2. Zinc concentrations (measured in nanomol per gram fresh weight ± SE) in the whole soft body of bivalves after exposure to Cd, Zn, or Cd and Zn.

Species	Metal exposure, nmol Zn g^{-1} (fw)			
	None	Cd	Zn	Cd + Zn
C. fluminea[a]	309 ± 34	323 ± 16	1067 ± 158[c]	696 ± 195[c]
D. polymorpha[a]	72 ± 20	98 ± 69	1326 ± 595[c]	1154 ± 249[c]
C. gigas diploid[b]	4165 ± 310	3871 ± 368	13499 ± 171[c]	15266 ± 955[c]
C. gigas triploid[b]	3424 ± 1042	4015 ± 797	9266 ± 847[c]	11006 ± 1540[c]

[a]*C. fluminea* and *D. polymorpha* were contaminated for 10 days. [b]Diploid or triploid *C. gigas* were contaminated for 14 days. [c]Significantly different to control ($P < 0.05$). Zinc determination was done in triplicate for each condition.

In *C. fluminea*, *D. polymorpha*, and diploid *C. gigas*, there was a slight decrease in Zn accumulation factor after Cd and Zn exposure compared to that obtained after a single Zn exposure.

CoxI gene expression profiles of control and metal contaminated bivalves were then compared by quantitative real-time RT-PCR.

Effects of cadmium on CoxI gene expression

As shown in Figure 1, our experiments demonstrated for the first time that *CoxI* gene expression was up-regulated by Cd in the three different species. However, the induction factors were different between each mollusk: although freshwater bivalves accumulated the same amount of Cd (Table 1), *CoxI* induction was 9.5-fold higher in Cd-exposed *D. polymorpha* than in Cd-exposed *C. fluminea* (Figure 1a). *CoxI* expression level also differed between diploid and triploid *C. gigas*, whereas the Cd concentration in tissues was similar: a 4.8 ratio of triploid versus diploid *CoxI* gene expression was observed (Figure 1b).

Effects of cadmium on CoxI gene expression in presence of zinc

In each species, Zn alone did not significantly affect *CoxI* gene expression level; indeed induction factors in *C. fluminea*, *D. polymorpha*, diploid *C. gigas*, and triploid *C. gigas* were 0.1 ± 0.1, 1 ± 0.2, 1.3 ± 0.3, and 0.1 ± 0.1, respectively. When Cd and Zn were present in the water column, *CoxI* gene expression level was increased and induction factors in *C. fluminea*, *D. polymorpha*, diploid *C. gigas*, and triploid *C. gigas* were equal to 6 ± 0.6, 4 ± 0.2, 2 ± 0.2, and 14 ± 2, respectively (Figure 1). Nevertheless, gene induction was significantly lower than after a single Cd contamination; it had decreased by 10- and 200-fold in *C. fluminea* and *D. polymorpha*, respectively (Figure 1a). *CoxI* gene expression was still higher in triploid oysters than in diploid (sevenfold) but *CoxI* gene induction level underwent a 3.5- and 2.4-fold decrease in diploid and triploid *C. gigas* (respectively) as compared with single Cd contamination (Figure 1b).

Discussion

We designed this study to establish a possible relationship between Cd contamination and expression of the mitochondrial gene *CoxI*. COXI is the subunit one of the Cytochrome *c* oxydase which provides a critical function during respiration by transferring electrons from Cytochrome *c* to oxygen and contributing to ATP generation (Malatesta *et al.* 1995). Indeed it has been demonstrated that a reduced level of COX activity causes functional reduction in Na^+–K^+-ATPase capacity, an important factor responsible for neuronal death in the mammalian brain (Greenamyre *et al.* 1999), and that complex IV inhibitors cause a rapid and severe depletion of cellular ATP content, resulting in acute cell death (Zhang *et al.* 2001).

In the present study, the first result of our experiments showed that *CoxI* gene expression was up-regulated in different aquatic organisms after Cd exposure. Overexpression of *CoxI* has been demonstrated associated with a pyrethroid insecticide resistant strain of *Blatella germanica* (German cockroach) (Pridgeon & Liu 2003) and in *Danio rerio* (zebrafish) fed with diets contaminated by methyl mercury (Gonzalez *et al.* 2005), but to our knowledge, the direct relation between *CoxI* expression and Cd contamination has never been reported before. Although *CoxI* gene induction by Cd is clear, no direct link was established between Cd concentration in tissues and *CoxI* gene expression level. Indeed, *CoxI* gene expression level was 10 times higher in *D. polymorpha* than in *C. fluminea*, whereas the Cd accumulation factor was lower in *D. polymorpha* than in *C. fluminea*. The finding of 4.8-times higher up-regulation of *CoxI* gene expression in triploid than in diploid *C. gigas* cannot be explained in a simple way by the presence of an extra structural gene copy, as a quantitative effect alone would be expected to be in the order of 1.5, or even less if the background expression is higher in the triploid due to the extra gene copy. The expression level of a single gene in a polyploid organism as compared to the diploid one is hardly predictable. For instance, in cotton (*Gossypium hirsutum*) the alcohol dehydrogenase A (*adhA*) gene expression level in tetraploid leaves is two times higher than in diploid ones whereas it is two times lower in tetraploid roots than in diploid ones. And the *adhD* gene is 10 times more expressed in the diploid leaves and roots than in tetraploid ones (Adams *et al.* 2003). This highlights the non-Mendelian mechanisms than often occurs for gene expressions in polyploid organisms.

As COX is considered as the rate-limiting step for mitochondrial respiration (Villani & Attardi 2000), *CoxI* gene overexpression could be a compensating mechanism to restore the decrease in mitochondrial activity and to efficiently consume O_2, thus limiting Cd induced damage in the cell. Some of this damage is a consequence of increased ROS production. Indeed, Cd stimulates ROS formation in mitochondria because it blocks electron transport, especially electron transfer through the complex III which is considered as the principal site for ROS generation (Chen *et al.* 2003; Wang *et al.* 2004). Prevention of the slowing electron flow can thus be considered as a mechanism to avoid ROS production. For instance, an increased activity of complex IV, causing the depletion of electron rich intermediates, could prevent Cd-promoted oxidative stress. Moreover, Cytochrome *c* oxydase activity has been shown to be higher in oxygen-tolerant HeLa cells than in the control cells (Campian *et al.* 2004).

Another result of this study showed that *CoxI* gene inducibility was lowered when bivalves were contaminated with Cd and Zn compared with single Cd contamination. Even for *D. polymorpha*, this reduction of *CoxI* gene expression level could not be explained only by a decrease in intracellular Cd accumulation. Indeed, the ratio of *CoxI* gene induction levels after Cd exposure versus after Zn + Cd exposure was 200 in *D. polymorpha* and 10 in *C. fluminea*, whereas the decrease in Cd accumulation was similar for the two species.

Whereas Cd induced ROS production, Zn was shown to have antioxidant properties. For instance, it protected cultured skin cells against the cytotoxic effects of UV irradiation and decreased the production of ROS (Parat *et al.* 1997). Moreover, Zn added to a cell culture medium inhibited Cd-induced ROS production (Szuster-Ciesielska *et al.* 2000). These findings suggest that at least one of the mechanisms involved in the protective role of Zn against Cd-induced toxicity is connected with inhibition of Cd-induced ROS formation in cells. In our study, the decreased *CoxI* gene expression level in bivalves contaminated with Cd and Zn compared to those contaminated only with

Cd could be explained by a lower toxic effect of Cd. In any case, *CoxI* gene expression level was reduced in the presence of Zn but remained at least two times higher than in control animals (Figure 1). Thus, *CoxI* gene expression level might constitute a new tool, in addition to metallothioneins and heat shock proteins levels, for detecting Cd contamination of both freshwater and marine sentinel organisms or for detecting Cd pollution from the river to the sea.

Acknowledgements

We would like to thank Gilles Durrieu for his help in statistical analysis.

References

Achard M, Baudrimont M, Boudou A, Bourdineaud J-P. 2004 Induction of a multixenobiotic resistance protein (MXR) in the Asiatic clam *Corbicula fluminea* after heavy metals exposure. *Aquatic Toxicol* **67**, 347–357.

Achard-Joris M, van den Berg van Saparoea HB, Driessen AJM, Bourdineaud J-P. 2005 Heterologously expressed bacterial and human multidrug resistance proteins confer cadmium resistance to Escherichia coli. *Biochemistry* **44**, 5916–5922.

Adams KL, Cronn R, Percifield R, Wendel JF. 2003 Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proc Natl Acad Sci USA* **100**, 4649–4654.

Bagchi D, Joshi SS, Bagchi M, *et al*. 2000 Cadmium- and chromium-induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL-60 cells, and normal human peripheral blood mononuclear cells. *J Biochem Mol Toxicol* **14**, 33–41.

Beyersmann D, Hechtenberg S. 1997 Cadmium, gene regulation, and cellular signalling in mammalian cells. *Toxicol Appl Pharmacol* **144**, 247–261.

Bray TM, Bettger WJ. 1990 The physiological role of zinc as an antioxidant. *Free Radical Biol Med* **8**, 281–291.

Campbell PGC. 1995 Interactions between trace metals and aquatic organisms a critique of the free-ion activity model. In: Tessier A, Turner DR, eds. *Metal Speciation and Bioavailability in Aquatic Systems*. John Wiley & Sons; Chichester: pp. 45–102.

Campian JL, Qian M, Gao X, Eaton JW. 2004 Oxygen tolerance and coupling of mitochondrial electron transport. *J Biol Chem* **279**, 46580–46587.

Capaldi RA. 1990 Structure and assembly of Cytochrome *c* oxidase. *Arch Biochem Biophys* **280**, 252–262.

Capaldi RA, Malatesta F, Darley-Usmar VM. 1983 Structure of Cytochrome *c* oxidase. *Biochim Biophys Acta* **726**, 135–148.

Carginale V, Capasso C, Scudiero R, Parisi E. 2002 Identification of cadmium-sensitive genes in the Antarctic fish *Chionodraco hamatus* by messenger RNA differential display. *Gene* **299**, 117–124.

Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. 2003 Production of reactive oxygen species by mitochondria: central role of complex III. *J Biol Chem* **278**, 36027–36031.

Company R, Serafim A, Bebianno MJ, Cosson R, Shillito B, Fiala-Medioni A. 2004 Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Mar Environ Res* **58**, 377–381.

Gonzalez P, Dominique Y, Massabuau J-C, Boudou A, Bourdineaud J-P. 2005 Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebra fish (*Danio rerio*). *Environ Sci Technol* **39**, 3972–3980.

Greenamyre JT, MacKenzie G, Peng TI, Stephans SE. 1999 Mitochondrial dysfunction in Parkinson's disease. *Biochem Soc Symp* **66**, 85–97.

Ikediobi CO, Badisa VL, Ayuk-Takem LT, Latinwo LM, West J. 2004 Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. *Int J Mol Med* **14**, 87–92.

Inza B, Ribeyre F, Maury-Brachet R, Boudou A. 1997 Tissue distribution of inorganic mercury, methylmercury and cadmium in the Asiatic clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment. *Chemosphere* **35**, 2817–2836.

Li ZS, Lu YP, Zhen RG, Szczypka M, Thiele DJ, Rea PA. 1997 A new pathway for vacuolar cadmium sequestration in Saccharomyces cerevisiae: YCF1-catalyzed transport of bis(glutathionato)cadmium. *Proc Natl Acad Sci USA* **94**, 42–47.

Malatesta F, Antonini G, Sarti P, Brunori M. 1995 Structure and function of a molecular machine: Cytochrome *c* oxidase. *Biophys Chem* **54**, 1–33.

Marigomez I, Soto M, Cajaraville MP, Angulo E, Giamberini L. 2002 Cellular and subcellular distribution of metals in molluscs. *Microsc Res Tech* **56**, 358–392.

Parat MO, Richard MJ, Pollet S, Hadjur C, Favier A, Beani JC. 1997 Zinc and DNA fragmentation in keratinocyte apoptosis: its inhibitory effect in UVB irradiated cells. *J Photochem Photobiol* **37**, 101–106.

Pridgeon JW, Liu N. 2003 Overexpression of the Cytochrome *c* oxidase subunit I gene associated with a pyrethroid resistant strain of German cockroaches, *Blattella germanica* (L.). *Insect Biochem Mol Biol* **33**, 1043–1048.

Roesijadi G, Hansen KM, Unger ME. 1996 Cadmium-induced metallothionein expression during embryonic and early larval development of the mollusc *Crassostrea virginica*. *Toxicol Appl Pharmacol* **140**, 356–363.

Szuster-Ciesielska A, Stachura A, Slotwinska M, *et al*. 2000 The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. *Toxicology* **145**, 159–171.

Turrens JF. 1997 Superoxide production by the mitochondrial respiratory chain. *Biosci Rep* **17**, 3–8.

Villani G, Attardi G. 2000 In vivo control of respiration by Cytochrome *c* oxidase in human cells. *Free Radical Biol Med* **29**, 202–210.

Waalkes MP, Coogan TP, Barter RA. 1992 Toxicological principles of metal carcinogenesis with special emphasis on cadmium. *Crit Rev Toxicol* **22**, 175–201.

Wang Y, Fang J, Leonard SS, Krishna Rao KM. 2004 Cadmium inhibits the electron transfer chain and induces reactive oxygen species. *Free Radical Biol Med* **36**, 1434–1443.

Yakes FM, Van Houten B. 1997 Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci USA* **94**, 514–519
.

Zhang J-G, Tirmenstein MA, Nicholls-Grzemski FA, Fariss MW. 2001 Mitochondrial electron transport inhibitors cause lipid peroxidation-dependent and -independent cell death: protective role of antioxidants. *Arch Biochem Biophys* **393**, 87–96.