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Cadmium *versus* copper toxicity: Insights from an integrated dissection of protein synthesis pathway in the digestive glands of mussel *Mytilus galloprovincialis*

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HIGHLIGHTS

- Cu^{2+}-exposure of mussels results in genotoxicity, without affecting MTs production.
- Cd^{2+}-exposure of mussels causes low genotoxicity, but induces MTs production.
- Both metals induce oxidative stress in mussels, with Cd being the strongest inducer.
- Translation is suppressed by both metals, mainly at the initiation and elongation steps.
- MTs abrogate translational defects caused by Cd^{2+}, by trapping the toxic metal.

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ABSTRACT

The main purpose of this study was to investigate the impact of metal-mediated stress on the protein-synthesis pathway in mussels. To this end, mussels (*Mytilus galloprovincialis*) underwent a 15 days exposure to 100 μg/L Cu^{2+} or Cd^{2+}. Both metals, in particular Cd^{2+}, accumulated in mussel digestive glands and generated a specific status of oxidative-stress. Exposure of mussels to each metal resulted in 40% decrease of the tRNA-aminoacylation efficiency, at the end of exposure. Cu^{2+} also caused a progressive loss in the capability of 40S-ribosomal subunits to form 48S pre-initiation complex, which reached 34% of the control at the end of exposure. Other steps of translation underwent less pronounced, but measurable damages. Mussels exposed to Cd^{2+} for 5 days presented a similar pattern of translational dysfunctions in digestive glands, but during the following days of exposure the ribosomal efficiency was gradually restored. Meanwhile, metallothionein levels significantly increased, suggesting that upon Cd^{2+}-mediated stress the protein-synthesizing activity was reorganized both quantitatively and qualitatively. Conclusively, Cd^{2+} and Cu^{2+} affect translation at several levels. However, the pattern of translational responses differs, largely depending on the capability of each metal to affect cytotoxic pathways in the tissues, such as induction of antioxidant defense and specific repair mechanisms.

1. Introduction

Although metals are natural constituents of all ecosystems, the huge increase in energy and mineral consumption has determined anthropogenic emissions of several metals, exceeding those from natural sources [1]. Contamination of soil and water with heavy metals poses a major environmental and human health problem [2]. Among these metals, some, such as cadmium (Cd), have no physiological function for cell and are toxic even at low concentrations. Others, like copper (Cu), are essential for cell, but can become toxic when their homeostasis is disrupted [3]. Reduced cell viability and function integrity could affect the health status of the organism exposed to metals [4].

All organisms, at least at cellular level, have the ability to respond to adverse environmental conditions, such as metal pollution, by activating an integrated stress response that reprograms metabolism and diverts anabolic energy into the repair of stress-induced molecular damage. A dynamical reprogramming of gene expression can occur at many different levels, ranging from the

Abbreviations: aa-tRNA, aminoacyl-tRNA; AcPhe-tRNA, acetylphenylalanyl-tRNA; LP, labilization period; MDA, malondialdehyde; MTs, metallothioneins; MN, micronucleus; PTase, peptidyl transferase; ROS, reactive oxygen species; TBARS, thiobarbituric reactive species; SOD, superoxide dismutase.

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accessibility of chromatin to the activity and stability of the produced proteins. Nevertheless, a major component of this stress response program targets the translational machinery [5]. This is often accomplished by modification of protein and RNA components of the translation machinery and/or by recruitment of specific mRNAs to sites of translation, storage or decay [6–8]. The profile of translational responses depends on the capability of each metal to switch the translational machinery to a selective synthesis of proteins that are required for cell survival under metal-induced stress. In bivalves mollusks, Cd^{2+} induces the selective biosynthesis of cytoprotective proteins, such as metallothioneins and heat shock proteins, which in turn scavenge free radicals [9–12]. In contrast, Cu^{2+} is unable to trigger metallothionein production [12,13].

The interference of Cu^{2+} and Cd^{2+} with total protein synthesis [11], polysome profile [12], and proteomic signatures in bivalves [14–17] has been already reported. On the other hand, many studies have been published on DNA damages and transcriptional changes in bivalves exposed to metals [18,19]. An important finding from these studies is that there is not always a good correlation between the production of a protein and the amount of mRNA coding this protein, a fact supporting the notion that post-transcriptional regulation of gene expression is of high significance.

Recently, we published on the dysfunctions of the translational machinery in digestive glands of mussels exposed to mercury ions [20]. The common mussel *Mytilus galloprovincialis* was chosen as a model organism in this study, given that one of the main reasons of human toxicity by metals is the consumption of seafood [21]. By functional analysis of each step of translation pathway, we revealed that regulation of protein synthesis by Hg^{2+} mainly occurs at the initiation and elongation steps of translation. However, one pattern does not fit at all. As shown in the present study, exposure of mussels to Cu^{2+} or Cd^{2+} results to a completely different profile of translational defects. The differentiated behavior of Cu^{2+} and Cd^{2+} as toxicants of the translational machinery is discussed in comparison with other data derived from literature.

2. Materials and methods

2.1. Exposure of mussels to metals

Mussels, *M. galloprovincialis*, of a very similar body weight (6.0 ± 0.3 cm shell length) were provided by a marine farm (Poseidon Co., Mandros; Galaxidi, Southern Greece). Mussels were in early gametogenesis, when bioaccumulated metals are least affected by body mass variations [22]. Mussels were kept unfed in tanks containing natural seawater, constantly aerated and changed every two days (salinity = 37–39‰; pH = 7.1–7.2; dissolved oxygen = 8.0–8.2 mg/L), to allow acclimation at 18 °C for 1 week, under natural photoperiod. Groups of 40 individuals per tank (40 L) were then exposed for up to 15 days to either 100 μg/L Cu^{2+} or 100 μg/L Cd^{2+}, added as chloride salts in natural seawater. During the exposure of mussels to metals, water change frequency and temperature were maintained the same as during the acclimation period, while 38 mg of food (PROCORAL PHYTON, Tropic Marin, Wartenberg, Germany) was added in each tank, daily, in two doses [20]. Metal addition was repeated after each change of water. Metal concentrations were sublethal ($<LC_{50}$), met by mussels residing in heavily polluted areas by metals [23–27], and often used in laboratory experiments in bivalves [28–32]. No mortality was recorded during the first 10 days of exposure, while at the 15th day of exposure the mortality was less than 5%. At the end of each exposure period, the mussels were collected and used either directly for the superoxide radical assay or their gills and digestive glands were dissected and prepared for further analysis.

2.2. Metal analysis

Metal measurement in seawater and digestive glands was performed by atomic absorption spectrophotometry, as previously described [13]. Merck standard solutions and certified reference materials (IAEA-436 tuna fish flesh homogenate) were used for calibration and verification of the accuracy of the analysis. Values obtained were consistently within the certified ranges. Standard deviation of five replicated samples was less than 7%. Metal concentration in seawater was expressed as μg/L, while in digestive glands as μg/g tissue (dry weight).

2.3. Biomarker analyses

Superoxide radical production was measured *in situ* according to Georgiou et al. [33] and expressed in pmol/mg tissue protein. Total proteins were determined in the tissue homogenate according to Bradford [34].

Lipid peroxidation in digestive glands was quantified as thiobarbituric reactive substances (TBARS) [35]. TBARS concentrations were calculated from an external standard curve of malondialdehyde (MDA) and the values were expressed as nmoles of MDA equivalents formed per mg tissue protein.

Analysis of superoxide dismutase (SOD) activity in digestive glands was based on the ability of SOD to inhibit the reduction of oxidized dianisidine by superoxide radical, and measured as described previously [12]. One unit of SOD was defined as the amount of SOD that inhibits the rate of dianisidine reduction by 50%.

Micronucleus (MN) frequency was measured in gill cells, as indicated by Bolognesi and Fenech [36]. Criteria used for the identification of micronuclei were adopted from the same reference.

Metallothionein (MT) content in digestive glands was measured indirectly, based on the detection of —SH residues obtained after ethanol/chloroform fractionation of the sample homogenates, according to Viarengo et al. [37]. The data were expressed in μg/g tissue (wet weight). assuming 21 cysteine residues per MT molecule and a molecular weight for *M. galloprovincialis* MT equal to 8600 Da.

Lysosomal membrane stability was determined by measuring the lysosomal permeability for the exogenous added substrate AS-BI *N*-acetyl-β-D-glucosaminidine of the lysosomal *N*-acetyl-β-hexosaminidase [38]. The permeability was assessed in 10-μm thick cryostat sections of mussel digestive glands by measuring the labilization period (LP), which is the preincubation time that is required to completely labilize the lysosomal membranes, at acid conditions and 37 °C.

2.4. Biochemical preparations

Total RNA, mRNA, tRNA, crude translation factors, and partially purified aminoacyl-tRNA (aa-tRNA) synthetases were prepared from the post-mitochondrial supernatant of digestive gland homogenates according to Pytharopoulou et al. [20]. Initiator $tRNA_i^{Met}$ was isolated from competent *Escherichia coli* cells, transformed with a pUC118 plasmid bearing the gene for yeast $tRNA_i^{Met}$ (kindly provided by Dr. Bruno Senger, IPCB-CNRS Strasbourg, France). [^{3}H]Phe-tRNA and [^{3}H]Met-$tRNA_i$ were prepared by reacting $tRNA^{Phe}$ (Sigma) and $tRNA_i^{Met}$, with [^{3}H]Phe and [^{3}H]Met, respectively, in the presence of ATP and partially purified aa-tRNA synthetases, according to Kalpaxis et al. [39]. Acetylation of [^{3}H]Phe-tRNA to acetyl[^{3}H]Phe-tRNA (Ac[^{3}H]Phe-tRNA) was performed under standard conditions [39]. A synthetic mRNA, mRNA-T, 5′-GUUCUCAGAAACUUAUGUGAAGUAUAGCAUCUAGAAAA-3′, carrying an initiation codon (AUG) that was followed by a termination codon (UGA), was prepared