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Metallothionein in the freshwater gastropod *Melanopsis dufouri* chronically exposed to cadmium: A methodological approach

Rocío Ureña [a], Maria João Bebianno [b], Jose del Ramo [a], Amparo Torreblanca [a,*]

[a] Department of Functional Biology, Faculty of Biological Sciences, University of Valencia, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain
[b] CIMA, Faculty of Marine and Environmental Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

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ABSTRACT

Previous studies have demonstrated that the use of differential pulse polarography (DPP) for metallothionein (MT) determination in marine gastropod tissues, particularly the digestive gland, requires taking into account the presence of heat-stable high molecular weight compounds that exhibit polarographic signal. In the present paper, similar compounds were identified in tissues from the freshwater snail *Melanopsis dufouri* which also interfere with MT determination by DPP and, due to their silver binding capacity, also interfere in the silver assay for MT quantification. Ultrafiltration seems to be effective in removing these high molecular weight compounds from heat-denatured homogenate supernatant allowing direct MT quantification by DPP. A fully validated procedure for metallothionein determination in *M. dufouri* is described. In spite of a considerable accumulation of cadmium in the visceral complex of *M. dufouri* following exposure to 100 μg Cd L^{-1} for 8 weeks (up to 37 μg g^{-1}) only a small increase in MT concentration was found.

1. Introduction

Molluscs have received great attention as sentinel organisms to pollutants both in marine and freshwater ecosystems (Flessas et al., 2000; Downs et al., 2001; Porte et al., 2001; Bebianno et al., 2004; Wepener et al., 2005). Their relatively limited mobility and higher availability are some of the advantages of the use of these animals in comparison to fish in pollution studies.

Metallothionein (MT) induction is one of the many biochemical responses that animals exhibit after being exposed to metals, particularly to Cd, and has been considered as a core biomarker in some biomonitoring programmes. Furthermore, MT has a pivotal role in metal detoxification by capturing the metal within the tissues in a non-toxic form.

Melanopsis dufouri is a common freshwater prosobranchia gastropod inhabiting most clean streams and rivers in the south and east of the Iberian Peninsula (Pujante, 1987; Pujante et al., 1998). The combination of metal pollution and low pH is leading to the disappearance of populations of this genus from the Guadiamar River, SW Spain, at metal impacted points (Sola et al., 2004). Although several works have been published concerning cadmium effects on freshwater gastropoda (Lam, 1996a, 1996b; Lam et al., 1997; Cheung and Lam, 1998; Lefcort et al., 2000; Cœurdassier et al., 2003), metal handling and detoxification has scarcely been studied in freshwater prosobranchia (Leung et al., 2003). As far as we know there are no available data on the effectiveness/potency of metallothionein induction by cadmium in this group. Reliable quantification of individual proteins is regaining interest as differential expression of proteins detected by genomic or proteomic approaches need to be confirmed by alternative methods such as antibody-based techniques or specific assays.

Many intercalibration exercises, comparative studies and reviews of different MT quantification techniques have been reported (Geret et al., 1998; Ivankovic et al., 2003; Dabrio et al., 2002; Zorita et al., 2005; Amiard et al., 2006). The discrepancies found among these methods highlighted the need to validate the method of choice in each species and/or tissue prior to routine MT determination. Metal saturation and polarography have been the most used methods for MT quantification in gastropoda (Table 1). However, previous studies have demonstrated that the use of differential pulse polarography for MT determination in some gastropod tissues requires the taking into account of the presence of heat-stable high molecular weight sulphydryl compounds which exhibit polarographic signals (Bebianno et al., 1992). Furthermore, the silver saturation method relies on the selective heat-stability of MT and on the high silver binding affinity to this protein and has been fully validated for measuring MT in mammals (Scheuhammer and Cherian, 1986) and crustaceans (Martínez et al., 1993) and may be suitable for measuring MT in gastropoda if chromatographic fractionation demonstrates a specific binding of silver to MT.

* Corresponding author. Fax: +34 6 3543202.
E-mail addresses: Rocio.Urena@uv.es (R. Ureña), mbebian@ualg.pt (M. João Bebianno), Juan.J.Ramo@uv.es (J. del Ramo), Amparo.Torreblanca@uv.es, torrebla@uv.es (A. Torreblanca).

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Table 1
Concentrations of MT found in the current work and in the literature for different aquatic gastropods, together with the determination method used. DPP refers to differential pulse polarography and TTM refers to the tetrathiomolybdate method. The units of MT are expressed in $\mu g\ g^{-1}$ wet weight, except those indicated with asterisks (*ng MT mg^{-1} protein; **fluorescence intensity). Wet to dry weight ratio was considered to be 4 for unit conversion. All of the metal exposures compiled here are for Cd unless otherwise specified. Those exposure conditions expressed as $\mu g\ g^{-1}$ refer to wet weight of food (lettuce).

Species	Tissue	Method	Origin	Values	Induction	Author
M. dufouri	Visceral complex	DPP	Control	1084.3		Current study
			6 $\mu g\ L^{-1}$	1089.5	No	
			100 $\mu g\ L^{-1}$	1474.4	No	
L. littorea	Digestive gland	DPP	Control	2500		Bebianno et al. (1992)
			4 $\mu g\ L^{-1}$	3375	No	
			40 $\mu g\ L^{-1}$	2000	No	
			400 $\mu g\ L^{-1}$	2650	No	
	Rest of tissues		Control	825		
			4 $\mu g\ L^{-1}$	800	No	
			40 $\mu g\ L^{-1}$	1125	Yes	
			400 $\mu g\ L^{-1}$	1000	Yes	
L. littorea	Gills	DPP	Control	587.5		Bebianno and Langston (1995)
			400 $\mu g\ L^{-1}$	1985	Yes	
	Kidney		Control	960		
			400 $\mu g\ L^{-1}$	2565	Yes	
P. aspera	Whole animal	DPP	Natural	1225–2450	No	Bebianno et al. (2003)
L. stagnalis	Whole animal	Ag saturation	Control	125		Leung et al. (2003)
			0.01 $\mu g\ L^{-1}$	150	No	
			1000 $\mu g\ L^{-1}$	375	Yes	
N. lapillus	Whole animal	Ag saturation	Control	192		Leung and Furness (1999a)
			500 $\mu g\ L^{-1}$	756	Yes	
	Branchia		Control	500		
			500 $\mu g\ L^{-1}$	650	No	
	Leiblein gland		Control	400		
			500 $\mu g\ L^{-1}$	1875	Yes	
	Kidney		Control	775		
			500 $\mu g\ L^{-1}$	2025	Yes	
	Digestive gland		Control	800		
			500 $\mu g\ L^{-1}$	900	No	
	Gonads		Control	875		
			500 $\mu g\ L^{-1}$	975	Yes	
L. littorea	Whole animal	Ag saturation	Natural	125–250		Leung and Furness (1999b)
N. lapillus	Leiblein gland	Ag saturation	Natural	100–500		Leung et al. (2001)
N. lapillus	Leiblein gland	Ag saturation	Control	270		Leung and Furness (2001a)
			500 $\mu g\ L^{-1}$	430	Yes	
N. lapillus	Leiblein gland	Ag saturation	Control	250		Leung and Furness (2001b)
			400 $\mu g\ L^{-1}$	1300	Yes	
N. lapillus	Digestive -gonads	Ag saturation	Control	22		Leung et al. (2002)
			500 $\mu g\ L^{-1}$	70	Yes	
	Leiblein gland		Control	52		
			500 $\mu g\ L^{-1}$	105	No	
N. lapillus	Whole animal	Ag saturation	Natural	12–62		Leung et al. (2005)
I. obsoleta	Whole animal	ELISA	Control	14*		Downs et al. (2001)
			560 $\mu g\ L^{-1}$	52*	Yes	
			5600 $\mu g\ L^{-1}$	70.2*	Yes	
H. trunculus	Hepatopancreas	Fluorescence Cu-MT	Natural	400**–1000**	Yes	Siboni et al. (2004)
M. tuberculata	Whole animal	Spectrophotometric	Natural	0.02–0.04	No	Wepener et al. (2005)
P. vulgata	Whole animal	Spectrophotometric	Control	30		Brown et al. (2004)
			6.1 g L^{-1} Cu	20	No	
H. pomatia	Digestive gland	Cd-chelex	control	294		Berger et al. (1995)
			36 $\mu g\ g^{-1}$	756	Yes	
C. hortensis	Digestive gland	Cd-chelex	Control	325		Dallinger et al. (2004a)
			9.2 $\mu g\ g^{-1}$	2925	Yes	
			64 $\mu g\ g^{-1}$	4550	Yes	
			Natural	130–1300	Yes	
H. pomatia	Digestive gland	Cd-chelex	1.2 $\mu g\ g^{-1}$	285		Chabicovsky et al. (2004)
			933 $\mu g\ g^{-1}$	2380	Yes	
H. pomatia	Digestive gland	Cd-chelex	Control	406		Dallinger et al. (2004b)
			68 $\mu g\ g^{-1}$ Cd	1382	Yes	
			132 $\mu g\ g^{-1}$ Cu	325	No	
	Mantle	TTM	Control	487.5		
			68 $\mu g\ g^{-1}$ Cd	406	No	
			132 $\mu g\ g^{-1}$ Cu	568	No	