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Thiol peptides induction in the seagrass *Thalassia testudinum* (Banks ex König) in response to cadmium exposure

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Abstract

Trace metal accumulation and thiol compounds synthesis as induced by cadmium exposure was studied in the seagrass *Thalassia testudinum*. Shoots were exposed for 24, 48, 96 and 144 h to several $CdCl_2$ concentrations (0, 30, 50 and 70 μM). Levels of cadmium, cysteine, glutathione (GSH), γ-glutamylcysteine (γ-EC), and phytochelatin-like peptides were determined in green blades, live sheaths and root/rhizomes tissues. Metal accumulation was dependent on Cd concentration and type of tissue, with green blades showing the highest content followed by live sheaths and root/rhizomes. All tissues experienced an increase in thiol-containing compounds as a response to cadmium exposure. Live sheaths showed the highest levels of cysteine, GSH and γ-EC. This is the first report of induction of thiol peptides, presumably phytochelatins, by a trace metal in a sea grass species.

Keywords: Sea grass; Cadmium; Thiol peptides; *Thalassia testudinum*; Phytochelatins

1. Introduction

Anthropogenic chemicals, such as heavy metals, represent one of the most toxic threats to seagrass meadows (Ward, 1989; Roméo et al., 1995; Schlacher-Hoenlinger and Schlacher, 1998). Seagrasses have showed great ability to accumulate several elements, and are increasingly used as a biological indicator of environmental quality (Pergent-Martini and Pergent, 2000; Pergent-Martini et al., 2005). Recent studies have focused on the use of early symptoms of stress, or biomarkers defined as "cellular, molecular and biochemical changes induced by chemical pollutants, measurable in biological systems such as tissues, cells and biological fluids" (Depledge et al., 1995). The principal biomarkers tested in plants are related to photosynthetic activity, enzyme activities, secondary metabolite synthesis, oxidative stress and detoxification mechanisms (Ferrat et al., 2003b). Phytochelatins (PCs) are synthesized by plants in response to heavy metal exposure, cadmium being the best activator known so far (Mendoza-Cozatl et al., 2005; Clemens, 2006). PCs are glutathione-derived peptides with a general structure (γ-Glu-Cys)*n*-Gly, where $n = 2–11$ depending on the species, type and level of metal exposure (Grill et al., 1985; Rauser, 1990; Cobett and Goldsbrough, 2002). Besides heavy metal induction, PCs may also be limited by GSH availability and, on the other hand, an excessive activity of PCs may lead to a GSH depletion causing oxidative stress to the cell (Mendoza-Cozatl and Moreno-Sanchez, 2006).

The impact of trace metals on sea grasses has been studied mainly by assessing the glutathione S-transferase (GST) activity as an indicator of trace metal stress in *Posidonia oceanica* (Hamoutène et al., 1996; Ferrat et al., 2000, 2002a,b, 2003a; Ranvier et al., 2000). It was suggested that phytochelatins were involved in the variable response of GST to oxidative stress. However, further analysis of PCs synthesis in seagrasses in response to trace metal stress has not been reported. Therefore, in the present work cadmium accumulation and thiol peptide induction in different tissues of the seagrass *Thalassia testudinum*, in response to Cd exposure, was evaluated.

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2. Materials and methods

2.1. Cadmium exposure

Samples of *Thalassia testudinum* were collected in a healthy meadow at Xahuayxol, in the southern region of Quintana Roo, Mexico. Samples were collected with a 150 mm diameter PVC corer; each core contained 20–25 shoots. Whole cores (including seagrass and sediments) were deposited in acrylic aquaria ($n = 48$) and filled with seawater for its transport to the laboratory within the following 2 h.

Forty-eight aquaria with sea grasses were randomly placed in a room with controlled temperature, and periods of light and darkness of 14:10 h for 72 h for acclimation, after which sea grasses were exposed to four $CdCl_2$ nominal concentrations: 0, 30, 50 or 70 μM; and four time exposure treatments of 24, 48, 96 or 144 h. Three replicates for each cadmium and time exposure treatment were used. Assays were carried out under static conditions, and the single dose of cadmium was added to the water column in each aquarium (10 l) at the beginning of the experiment. At the end of the experiments in each aquaria five shoots were randomly selected for biomarker and cadmium analysis.

2.2. Cadmium accumulation

Material adhering to the external surfaces of plant tissues (e.g. epiphytes) was removed by scraping with a PVC scraper (Pergent-Martini, 1998). All samples were then rinsed three times with deionised water. Three tissues were obtained from each shoot: leaves, that were dissected into green blades and live sheaths, and roots/rhizome. Three replicates for each tissue and treatment were analyzed. Each tissue was freeze-dried and 0.5 g dry weight of independent samples was digested with 5 ml 70% nitric acid and 2 ml 72% hydrogen peroxide. The mineralized samples were adjusted to 25 ml with deionised water and analyzed with an atomic absorption spectrophotometer (Perkin-Elmer 3110). The analytical procedure was checked using a standard reference material (Lagarosiphon major, Nr. 60) from the Community Bureau of Reference (Commission of the European Community), with an average recovery of 94.5%.

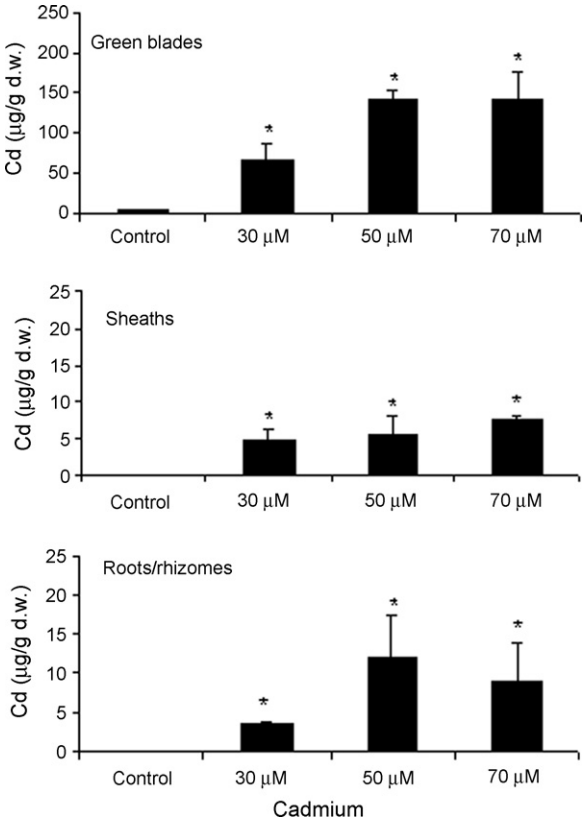

Fig. 1. Means ± S.D. ($n = 3$) of cadmium accumulation in different tissues of the seagrass *Thalassia testudinum* exposed for 96 h to 0 μM (control), 30 μM, 50 μM and 70 μM of $CdCl_2$. Asterisk represents significant differences with respect to control ($p < 0.05$, K–W ANOVA).

2.3. Extraction of thiol-compounds

Green blades, sheaths and root/rhizomes were lyophilized and homogenized in small mortars and pestles with liquid nitrogen and 1 ml of 50 mM Tris–HCl buffer (pH 8) containing 1 mM EGTA. Homogenates were centrifuged at 50,000 × *g* at 4 °C for 20 min. Thiol-compounds were reduced by incubating the super-

Table 1
Kruskal–Wallis non-parametric analysis of variance for total Cd accumulation and total thiol peptide content in green blades, sheaths and root/rhizome of *Thalassia testudinum*

Exposure conditions	Total Cd^{2+}	Total thiol-compounds	PC2-like thiol peptides
Green blades			
24 h	$H = 7.23$ $p = 0.065$	$H = 6.17$ $p = 0.104$	$H = 5.17$ $p = 0.160$
48 h	$H = 8.08^*$ $p = 0.044$	$H = 3.50$ $p = 0.321$	$H = 4.17$ $p = 0.244$
96 h	$H = 9.36^*$ $p = 0.025$	$H = 3.36$ $p = 0.339$	$H = 4.27$ $p = 0.234$
144 h	$H = 8.13^*$ $p = 0.043$	$H = 5.56$ $p = 0.135$	$H = 2.59$ $p = 0.459$
Sheath			
24 h	$H = 8.72^*$ $p = 0.033$	$H = 5.91$ $p = 0.116$	$H = 4.81$ $p = 0.186$
48 h	$H = 6.85$ $p = 0.077$	$H = 5.91$ $p = 0.116$	$H = 3.00$ $p = 0.392$
96 h	$H = 8.87^*$ $p = 0.031$	$H = 4.16$ $p = 0.245$	
144 h	$H = 9.15^*$ $p = 0.027$	$H = 1.67$ $p = 0.644$	$H = 2.83$ $p = 0.419$
Root/rhizome			
24 h	$H = 7.23$ $p = 0.065$	$H = 0.47$ $p = 0.925$	$H = 2.36$ $p = 0.502$
48 h	$H = 7.69$ $p = 0.053$	$H = 0.89$ $p = 0.828$	$H = 4.12$ $p = 0.249$
96 h	$H = 9.02^*$ $p = 0.029$	$H = 3.17$ $p = 0.367$	$H = 4.03$ $p = 0.259$
144 h	$H = 6.99$ $p = 0.072$	$H = 4.82$ $p = 0.185$	$H = 5.36$ $p = 0.147$

* Significant difference with respect to control.