Marine Pollution Bulletin 64 (2012) 2453–2460

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Kandelia obovata (S., L.) Yong tolerance mechanisms to Cadmium: Subcellular distribution, chemical forms and thiol pools

Bosen Weng [a], Xiangyu Xie [a], Dominik J. Weiss [a,b], Jingchun Liu [a], Haoliang Lu [a], Chongling Yan [a,*]

[a] Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, School of Life Sciences, Xiamen University, Xiamen 361005, PR China
[b] Department of Earth Science and Engineering, Imperial College London, London SW72AZ, United Kingdom

ARTICLE INFO

Keywords:
Kandelia obovata (S., L.) Yong
Cadmium
Tolerance mechanisms
Thiol pool
Subcellular distribution
Chemical form

ABSTRACT

In order to explore the detoxification mechanisms adopted by mangrove under cadmium (Cd) stress, we investigated the subcellular distribution and chemical forms of Cd, in addition to the change of the thiol pools in *Kandelia obovata* (S., L.) Yong, which were cultivated in sandy culture medium treated with sequential Cd solution. We found that Cd addition caused a proportional increase of Cd in the organs of *K. obovata*. The investigation of subcellular distribution verified that most of the Cd was localized in the cell wall, and the lowest was in the membrane. Results showed sodium chloride and acetic acid extractable Cd fractions were dominant. The contents of non-protein thiol compounds, Glutathione and phytochelatins in *K. obovata* were enhanced by the increasing strength of Cd treatment. Therefore, *K. obovata* can be defined as Cd tolerant plant, which base on cell wall compartmentalization, as well as protein and organic acids combination.

1. Introduction

Cadmium (Cd) is a ubiquitous toxic trace pollutant, which enters the environment mainly through air pollutants and soil applications of commercial fertilizers, sewage sludge and industrial processes (Adams et al., 2004; Mann et al., 2002). It could be toxic to living cells even at very low concentrations. Plants affected by Cd showed impaired photosynthesis, mineral nutrition deficiency, water imbalance and accelerated senescence (di Toppi and Gabbrielli, 1999). Meanwhile, Cd can be highly accumulated in plant tissues and transferred to the food chain due to its high soil–plant mobility. Therefore, it has turned out to be a serious threat and has become a major public concern (Mann et al., 2002; Salt et al., 1995).

Recent researches have focused on the accumulation and tolerance mechanisms of heavy metals in plants. These mechanisms involved internal metal detoxification processes, which may be achieved through complexation with cellular ligands such as non-protein thiol compounds (NPT, mainly including phytochelatins and glutathione), organic acids, cysteine and other low molecular weight thiols (Hernandez-Allica et al., 2006; Kupper et al., 2004; Schat et al., 2002). Non-essential metals accumulated in the cell wall and vacuole is another detoxification process, which prevented them from entering more sensitive cell metabolic sites.

NPT can be found in most plants, microorganisms and all mammalian tissues. They play a key role in the regulation of redox balance and can be used as indicators of oxidative stress in the detoxification process against xenobiotics and heavy metals (Marrs, 1996). Phytochelatin (PCs) are synthesized from glutathione (GSH) with the structure of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ ($n = 2–11$), which are thiol-containing peptides that take part in heavy metal detoxification due to their capability of binding metal ions inside the cells (Cobbett, 2000; Grill et al., 1985). Several studies have revealed that PCs synthesis is activated both in vivo and in vitro by a wide range of metal ions, including Cd^{2+}, Cu^{2+}, Pb^{2+}, Ag^{+}, Zn^{2+} and Hg^{2+} (Rauser, 1995; Zenk, 1996). PCs in higher plants are considered to be an important detoxification mechanism against Cd toxicity (Rauser, 1995). GSH plays an important role in maintaining reducing conditions inside cells and in protecting plants from environmental stress, including oxidative damage and excess xenobiotic organic compounds or heavy metals. Many studies have demonstrated that phytoplankton species can respond to metal toxicity through the production of antioxidant compounds (Pinto et al., 2003) and intracellular metal-binding thiol peptides (Kawakami et al., 2006). A study on *Kandelia candel* and *Bruguiera gymnorrhiza* treated with metals suggested that increased contents of proline, GSH and PCs in these plants might be associated with the efficiency of these antioxidants involved in metal tolerance (Huang and Wang, 2010).

Abbreviations: K. obovata, *Kandelia obovata* (S., L.) Yong; NPT, non-protein thiol compounds; PCs, phytochelatins; GSH, glutathione; CK, blank control.

* Corresponding author. Tel.: +86 592 2183805; fax: +86 592 2188846.
E-mail address: ycl@xmu.edu.cn (C. Yan).

0025-326X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2012.07.047

Regionalization of cell wall deposition and vacuolar compartmentation plays a major role in heavy metal detoxification, tolerance and hyperaccumulation in plants. Cell vacuole is considered to be the organ with greatest potential of Cd accumulation (Vogeli-Lange and Wagner, 1990). Cadmium is enriched mainly in the cell wall of root epidermal and mesophyll cell vacuoles of hyperaccumulator *Thlaspi caerulescens* and *Arabidopsis halleri* (Boominathan and Doran, 2003; Kupper et al., 2000). Chemical forms of Cd obtained from different extractants indicated the complex forms and mobility of Cd. This suggests that chemical forms could be one of the most important heavy metal detoxification mechanisms; it is also proven by the study of Cd chemical forms in *Bechmeria nivea* (L.) Gaud (Wang et al., 2008).

Mangrove ecosystems are one of the major types of habitats found in the intertidal zone, commonly situated in tropical, subtropical and temperate coastal zones, and are recognized as a massive sink of heavy metal pollutants, such as Zn, Cu, Cd, Pb. Many studies have suggested that mangroves possess a remarkable capacity to retain heavy metals, and tolerate relatively high levels of heavy metal pollution (Soto-Jiménez and Páez-Osuna, 2001). As one of the most dominant mangrove species along the south China coast, *K. obovata* has been widely studied. Previous studies were mainly concentrated on the accumulation of heavy metals, effect of heavy metals on plant growth, and effects of root exudates on heavy metal toxicity (Lu et al., 2007; Xie et al., 2012). However, to our knowledge, few articles have reported on the important mechanisms involved in the metal uptake and tolerance of mangrove plants.

The aims of this study were to characterize the translocation of Cd from roots to shoots in *K. obovata*, and to determine the content, subcellular distribution, and chemical forms of Cd in *K. obovata* and their roles in Cd tolerance. To evaluate the role of the thiol pools in tolerating Cd toxicity, NPT, glutathione, and phytochelatins were also determined. A possible Cd binding peptide in *K. obovata* was recognized by size exclusion chromatography coupled with ICP-MS (UV-SEC-ICP-MS).

2. Materials and methods

2.1. Plant materials and culture conditions

Mature *K. obovata* propagules were collected from the Jiulongjiang Estuary in March 2007. Only complete, undamaged propagules with intact testa and no emergent hypocotyls or radicles were selected for planting. The fresh weights of the selected propagules were 19.5 ± 1.2 g (Mean ± S.D.). They were planted in plastic pots (35 cm in diameter × 15 cm in depth) with sandy culture medium. Seedlings were watered with 1/4 Hoagland nutrient solution (twice a month) and tap water during the growing season when necessary. All the treated plants were placed under greenhouse conditions with natural light, day/night temperatures of 33/25 °C, and day/night humidity of 65/85%. After three months, healthy seedlings were chosen for experimentation. All seedling heights were 331 ± 20 mm, and leaf number was 8 ± 0.71 leaves.

2.2. Laboratory processing and sampling strategy

To each individual treatment group (with three replicates), an appropriate solution of the metal salt was applied to arrive across the concentration ranges, respectively at: 0 (CK), 2.5, 5, 10, 20, 40, 60 mg L^{-1}. The Cd treatment lasted for a month and the Cd solution was replaced once a week during the whole process. Thiol compounds were determined at 7 d and 30 d (at harvest) after Cd application. At harvest, plant roots were soaked in 20 m mol L^{-1} Na_2-EDTA for 15 min to remove metal ions adhering to the root surface (Yang et al., 1996). Then the whole plant were washed with distilled water, and were separated into leaves, stems, hypocotyls, and roots.

2.3. Extraction and determination of NPT and GSH

The NPT content of the plants was measured according to Del Longo et al. (1993). GSH (reduced) was estimated fluorimetrically according to Hissin and Hilf (1976).

2.4. Cd subcellular distribution extraction procedure

To determine what proportion of the metal was bound to, a heavy metal extraction procedure was carried out according to the method described by LozanoRodriguez et al. (1997). Leaf and root Cd subcellular distribution were divided into 4 fractions respectively: cell wall-bound Cd (FA), organelles-bound Cd (FB), membrane-bound Cd (FC), soluble Cd (FD). All fractions, except for the FD, were rinsed in redistilled water four times, then dried at 105 °C for 24 h to constant weight for the acid digestion later.

2.5. Extraction of Cd in different chemical forms

Six chemical forms of Cd were extracted step by step using a sequence of designated extractants in the following order (Wang et al., 2008; Wu et al., 2005a; Yang et al., 1995):

(1) Eighty percent ethanol, extracting inorganic Cd, which include nitrate/nitrite, chloride, and aminophenol Cd,
(2) deionized water, extracting water-soluble Cd of organic acid complexes and $Cd(H_2PO_4)_2$,
(3) one mol L^{-1} NaCl, extracting Cd integrated with pectate and protein,
(4) 2% acetic acid (HAC), extracting insoluble $CdHPO_4$ and $Cd_3(PO_4)_2$ and other Cd-phosphate complexes,
(5) 0.6 mol L^{-1} HCl, extracting oxalate acid bound Cd,
(6) Cd in the residue.

A total of 150 mL supernatant of each five extraction solutions and the residue were evaporated to constant weight separately in an oven at 70 °C.

2.6. Extraction and determination of the binding peptide

Leaves and roots (20 g) of *K. obovata* were ground to powder under liquid nitrogen. The powder was extracted three times fully with 8 mL protein extracts (0.2 mol L^{-1} Tris–HCl, 1 mmol L^{-1} PMSF, 1 mmol L^{-1} EDTA, 1 mmol L^{-1} SDS, and 20 mmol L^{-1} mercaptoethanol, adjusted to pH 8.0 by 6 mol L^{-1} HCl). All the extracting solution was then refrigerated centrifuged at 20,000×*g* for 15 min. The supernatant was precipitated by adding 85% $(NH_4)_2SO_4$ and then stirred 4 h in a magnetic stirring apparatus. The solution obtained was refrigerated centrifuged at 40,000×*g* for 10 min. The supernatant was freeze-dried, and then redissolved in 3 mL Tris–HCl (50 mmol L^{-1}, pH 8.0) and refrigerated centrifuged at 10,000×*g* for 10 min. After that, the supernatant was filtered through 0.45 μm ultrafiltration membrane and analyzed by UV-SEC-ICP-MS (Lai et al., 2006; Sugaya et al., 2000).

2.7. Cd concentration analysis

Different parts of the plant were dried at 105 °C for 24 h to constant weight. After that, all plant parts, subcellular distribution fractions (except for the FD), and six chemical form components were weighed to determine the dry weight (DW) and then wet digested in concentrated HNO_3:H_2O_2 (5:1, v:v) at 140 °C for four