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The importance of glutathione and phytochelatins on the selenite and arsenate detoxification in *Arabidopsis thaliana*

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ABSTRACT

In this study, we investigated the role of glutathione (GSH) and phytochelatins (PCs) on the detoxification of selenite using *Arabidopsis thaliana*. The wild-type (WT) of *Arabidopsis thaliana* and its mutants (glutathione deficient Cad 2–1 and phytochelatins deficient Cad 1–3) were separately exposed to varying concentrations of selenite and arsenate and jointly to both toxicants to determine their sensitivities. The results of the study revealed that, the mutants were about 20-fold more sensitive to arsenate than the WT, an indication that the GSH and PCs affect arsenate detoxification. On the contrary, the WT and both mutants showed a similar level of sensitivity to selenite, an indication that the GSH and PCs do not significantly affect selenite detoxification. However, the WT is about 8 times more sensitive to selenite than to arsenate, and the mutants were more resistant to selenite than arsenate by a factor of 2. This could not be explained by the accumulation of both elements in roots and shoots in exposure experiments. The co-exposure of the WT indicates a synergistic effect with regards to toxicity since selenite did not induce PCs but arsenic and selenium compete in their PC binding as revealed by speciation analysis of the root extracts using HPLC–ICP–MS/ESI–MS. In the absence of PCs an antagonistic effect has been detected which might suggest indirectly that the formation of Se glutathione complex prevent the formation of detrimental selenopeptides. This study, therefore, revealed that PC and GSH have only a subordinate role in the detoxification of selenite.

Introduction

Selenium is known for its roles in biological systems because of its antioxidant and pro-oxidant properties (Mezes and Balogh, 2009). Apart from its role as a growth elicitor and abiotic stress tolerance enhancer, (Mezes and Balogh, 2009) it has also been found to reduce the toxic effects of other elements like cadmium, mercury, lead, antimony and arsenic in plants (Srivastava et al., 2009; Sun et al., 2010; Malik et al., 2012; Filek et al., 2008; He et al., 2004; Shanker et al., 1996; Feng et al., 2011). However, at concentrations above 20 μmol/L selenium has been found to induce toxic effects in plants. The toxic effects of selenium, which include stunted growth, chlorosis, leaves withering, decreased protein synthesis and death of plants (Terry et al., 2000; Hawrylak and Szymańska, 2004; Martin, 1936) are similar to toxicity symptoms from other toxic elements like cadmium, mercury, lead and arsenic (Spallholz and Hoffman, 2002; Mascher et al., 2002; Hartley-Whitaker et al., 2001; Hall, 2002) which suggest similar biochemical interactions in the plants. Most of the toxic

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effects of elements like cadmium, mercury, antimony, lead and arsenic, have to do with the generation of reactive oxygen species which damage cellular functions and are closely linked with their interactions with catalytically active thiols (Hawrylak and Szymańska, 2004; Hall, 2002; Sharma and Dietz, 2009; Gill and Tuteja, 2010; Stohs and Bagchi, 1995). For selenium in its oxyanion forms (selenite and selenate), its toxic effects are thought to be due to its non-specific incorporation in the plant's protein body by replacing sulphur in cysteine and methionine (Terry et al., 2000). However, toxic effects due to its interaction with essential sulfhydryl-containing enzymes and structural proteins leading to oxidative stress at high concentrations have also been widely reported (Mezes and Balogh, 2009; Hawrylak and Szymańska, 2004; Spallholz and Hoffman, 2002; Hartkainen et al., 2000; Chen et al., 2007; Spallholz, 1994, 1997). The first indication of the interaction of selenium with thiols leading to its toxic effects already reported in 1941 (Painter, 1941) and was later corroborated by Ganther (1968), who elucidated the reaction of selenite with glutathione.

Generally speaking, plants have developed different mechanisms for toxic element detoxification and tolerance (Hall, 2002; Zenk, 1996; Memon et al., 2001). The most common tolerance and detoxification mechanisms used by plants for toxic metals and metalloids involve the activation of a self-regulatory and inactivating enzyme commonly known as phytochelatin (PC) synthase (Memon et al., 2001; Ha et al., 1999; Loeffler et al., 1989). Phytochelatins are a class of metal/metalloid binding peptides formed by a wide variety of plant species in response to uptake of toxic metals/metalloids (Ha et al., 1999; Maitani et al., 1996; Ogawa et al., 2011). They are known for detoxification of toxic elements in plants and have a basic structure (ɣ-Glu-Cys)*n*-Gly, where *n* is in the range 2–11 but typically *n* = 2–4 (Wood and Feldmann, 2012). The terminal glycine can also be replaced with serine, alanine and glutamate (Maitani et al., 1996; Cobbett, 2000).

The mechanisms of plant tolerance to selenium oxyanions (common selenium forms in the soil) are not established, and in particular with regard to the roles of glutathione (GSH) and PC. Even though GSH undoubtedly plays significant roles in plant detoxification systems (Dixon et al., 1998; Guo et al., 2008), there have been contradicting reports on the activation/induction of GSH and PCs by selenite in plants. Grill et al. (1987) reported the induction of PCs by a wide variety of metal ions including selenite in cell suspensions of *Rauvolfia serpentina*, another study later reported that selenite did not induce PC production (Maitani et al., 1996; Zenk, 1996).

The reason for the contradictory results between Grill et al. and Zenk et al. might be as a result of different analytical methods used. Whilst Grill et al. used high-performance liquid chromatography (HPLC), Zenk et al. used extended X-ray absorption fine structure (EXAFS). The presence of various complexes of GSH with selenium in a selenized yeast extract (Dernovics and Lobinski, 2008a, 2008b; Lindemann and Hintelmann, 2002) and incubation of end-capped PC2 with selenite leading to formation of a stable selenotrisulfide (Spain and Rabenstein, 2004) suggest the possibility of formation of selenium-PC complex(es) in plants. The first identification of selenium-PC complex (Se^{II}-PC2) in plants was reported by our group in previous studies on *Thunbergia alata*, a plant which is known to produce PCs at low levels of exposure to arsenate (Bluemlein et al., 2009a, 2009b, 2009c; Aborode et al., 2015).

Factors that favour accumulation or uptake and translocation of selenium in plants need to be understood because of its essentiality in human diets. Therefore, in order to gain a better understanding about the roles of GSH and PCs in selenium detoxification in plants, we used *Arabidopsis thaliana*. The Columbia wild-type (WT) was used as a reference plant for selenite and arsenate sensitivity tests, and its derivative mutants were used to confirm the role of GSH and/or PCs in selenium detoxification. One of the mutants is a GSH-deficient mutant Cad 2–1 (Howden et al., 1995a; Meinke and Koornneef, 1997), which is cadmium sensitive owing to the decrease in its ability to biosynthesis GSH, which is about 15% to 30% of that of the WT (Howden et al., 1995a). The other mutant used in this study is a PC deficient mutant Cad 1–3, which has its PC synthase gene deleted and hence has lost its capacity to produce PCs. It has a PC synthesis capacity of about 8% of that of the WT and hence its hypersensitivity to cadmium despite having almost the same level of GSH (Howden et al., 1995b). Both Cad 1–3 and Cad 2–1 have been used to prove the role of PCs in metal/metalloid detoxification (particularly for cadmium and arsenic) (Howden et al., 1995b; Liu et al., 2010). Since PCs are known to be about 1000 fold more effective than GSH in metal detoxification and reactivation of poisoned enzymes (Kneer and Zenk, 1992), the Cad 1–3 mutant is particularly important in this study because it allows a direct test of the hypothesis regarding the role of PCs in the selenium detoxification mechanism.

The aims of this study, were (i) to investigate whether GSH and/or PCs play any role in selenium detoxification using the sensitivities of the mutants (Cad 2–1 and Cad 1–3) in comparison with that of the WT to selenite, (ii) to investigate whether selenium induces the production of PCs and compare this to the arsenate exposure and (iii) to investigate if there is competition between selenium and arsenic for PCs when they are co-exposed to plants and hence the detoxification or otherwise of selenite in the presence of arsenate. Selenite and arsenate were chosen because they are the most abundant species of these elements in aerobic soil.

1. Materials and methods

1.1. Chemicals and reagents

All chemicals used were of analytical grade or better. Deionised water (18 MΩ cm) was used throughout (Elga UK). Formic acid (90%), methanol (HPLC grade) and hydrogen peroxide (32%) were from Fisher Scientific UK. Potassium sulphate and sodium dihydrogen orthophosphate dihydrate were supplied by BDH. Gallium used as internal standards was from High Purity Standards, Charleston (USA). Magnesium sulphate heptahydrate, calcium nitrate tetrahydrate and potassium nitrate were supplied by Sigma Aldrich (UK). Sodium selenite (Se(IV)) and sodium hydrogen arsenate heptahydrate (As(V)) was obtained from Alfa Aesar (Germany). Sodium dimethylarsinic acid (98%) used as calibration standard for inductively coupled plasma mass spectrometry (ICP–MS) was obtained from ChemService (USA). Nitric acid

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