Microchemical Journal 98 (2011) 163–169

Contents lists available at ScienceDirect

Microchemical Journal

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

Stability of arsenic species in hydroponic media and its influence on arsenic uptake and distribution in White mustard (*Sinapis alba L.*)

Lukasz Jedynak, Joanna Kowalska *

Chemistry Department, Warsaw University, Pasteura 1, 02-093 Warsaw, Poland

ARTICLE INFO

Article history:
Received 15 October 2010
Received in revised form 5 January 2011
Accepted 5 January 2011
Available online 20 January 2011

Keywords:
Arsenic
Speciation
White mustard
Phytochelatins
Hydroponic culture

ABSTRACT

The long-term uptake and distribution of arsenic compounds by hydroponically cultivated White mustard (*Sinapis alba*) was investigated with a special emphasis on controlling the stability of the arsenic compounds in nutrient solution during the experiment. It was concluded that arsenites are rapidly oxidised to arsenates during the 7-day cultivation of White mustard. The presence of plant roots increases the oxidation rate of arsenites. Dimethylarsinic acid (DMA) and arsenates remain stable during the exposition, while monomethylarsonic acid (MMA) is partially demethylated. When the nutrient solution containing arsenites is exchanged daily, the distribution of arsenic in White mustard is significantly different (translocation factor—TF—is 70 times higher) in comparison to the experiment without exchange of the medium. Speciation analysis of arsenic in plant tissues and in nutrient solutions was performed by high performance liquid chromatography with inductively coupled plasma-mass spectrometry (HPLC ICP-MS). The results obtained unquestionably illustrated that the uncontrolled conditions of hydroponic plant cultivation may be a source of misinterpretation of all the obtained data. Additionally, the synthesis of phytochelatins in plants exposed to different arsenic compounds was investigated. Phytochelatins were identified in tissues of plants exposed to arsenites and arsenates, and their presence was correlated with high arsenite content. Phytochelatin synthesis was not indicated in plants grown in the presence of MMA and DMA.

1. Introduction

Arsenic is an element of interest due to the toxic properties of several arsenic compounds. Toxic arsenic compounds (mainly arsenites and arsenates) may exist in the environment due to natural processes, but the main source of pollution is anthropogenic activities (e.g., mines) [1,2]. Quite often, studies on hydroponically cultivated plants are the first step in investigations on plant species with potential for decontaminating soils [3–5].

Phytoremediation is an environmentally friendly approach that may be used to cope with environmental contamination. Plant species with the potential for phytoremediation should be characterised by relatively high biomass, fast biomass increments, and a bioconcentration factor (BF) and translocation factor (TF) above 1 [6]. There are several plant species defined as arsenic hyperaccumulators that could potentially be used to decontaminate arsenic-polluted soils [7–10]. Many other species were investigated focusing on their use as phytoextractors of arsenic-contaminated sites [11–14].

In studies on arsenic uptake by hydroponically cultivated plants, usually not enough attention is focused on the stability of the arsenic species in the nutrient medium [15–17]. Only a minority of publications in this field contain information about controlling arsenic speciation in the medium during the experiment [18–21].

It should be also stressed that studies on arsenic uptake by hydroponically cultivated plants are often carried out only for arsenates [22–25]. Moreover, usually only the short-term uptake (up to 24 h) is investigated [26,27].

Despite the growing amount of publications focusing on arsenic uptake and arsenic speciation in plant tissues, there are still many questions and doubts concerning the mechanisms of arsenic detoxification and its metabolism in terrestrial plants [28,29]. One of the most widespread arsenic detoxification mechanisms occurring in terrestrial plants is the complexation of arsenic with phytochelatins. There are many terrestrial plant species utilising this detoxification mechanism [30–34]. However, there are still some gaps that need to be filled [35].

In our previous studies on White mustard [36], we compared the concentration and speciation of arsenic incorporated by plants grown in the presence of different arsenic compounds. The results indicated a strong influence of the form of arsenic present in the nutrient solution upon the arsenic uptake and speciation in plant organs. A range of arsenic species were identified in the plants studied despite being grown in nutrient solutions containing a single form of As; however, the speciation of arsenic compounds in nutrient solutions was not controlled. In the present study, a long-term uptake and distribution of four arsenic compounds by hydroponically cultivated White

* Corresponding author. Tel.: +48 22 8220211x241; fax: +48 22 8220211x341.
E-mail address: askow@chem.uw.edu.pl (J. Kowalska).

0026-265X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2011.01.004

mustard (*Sinapis alba*) was investigated with a special emphasis on controlling the stability of the arsenic compounds in the medium during the experiment. Arsenic speciation in plant tissues was investigated as well. Moreover, an additional task of the investigation was to answer which arsenic compounds lead to the synthesis of phytochelatins in plant tissues.

2. Materials and methods

2.1. Cultivation of White mustard

Seeds of White mustard were soaked in deionised water for 1 h, placed in boxes on wet blotting paper, and incubated for 4 days. The seedlings were then placed in pearlite. Plants with developed root systems were transferred to boxes containing Knopp solution and the appropriate arsenic compounds. One litre of the nutrient solution contained 500 mg of $Ca(NO_3)_2{\cdot}4H_2O$, 150 mg of KNO_3, 150 mg of $MgSO_4$, 150 mg of KH_2PO_4, 8 mg of FeNaEDTA, 0.482 mg of $MnSO_4{\cdot}4H_2O$, 0.575 mg of $ZnSO_4{\cdot}7H_2O$, 1.25 mg of $CuSO_4{\cdot}5H_2O$, and 18.5 μg of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. Tris(Hydroxymethyl)aminomethane (1 mL of 1 mol L^{-1} Tris per 1 L of nutrient solution) was added to adjust the pH to 6.0–6.5. The plant cultivation was carried out in a growth chamber at 20 °C/16 °C (14 h day/10 h night). Simultaneously, additional plants were cultivated in the nutrient solution without added arsenic. Nutrient solutions in all the vessels were aerated continuously. The harvested plants were washed in milli-Q water and divided into leaves, stems and roots. A portion of the plant material dedicated to the total arsenic determination and speciation analysis was dried at 60 °C in a laboratory dryer. Preparation of the samples for the analysis of thiols was carried out using fresh plant material.

2.2. Long-term uptake of arsenic compounds (Experiment 1)

The first experiment was carried out to assess the long-term uptake and transport of 4 arsenic compounds by White mustard and to confirm if plants synthesise phytochelatins in response to stress caused by arsenic compounds. The plants were placed in 1-L vessels (3 plants per vessel) and grown for 3 weeks in nutrient solutions containing 1 mg L^{-1} of As(III), As(V), DMA and MMA, respectively. The experiment for each arsenic form was performed in triplicate. Nutrient solutions were refilled every 2–3 days. After 3 weeks, the plants were harvested, and the determination of the total arsenic and thiols in the plant tissues was performed.

2.3. Arsenic uptake by plants in the later stages of their growth (Experiment 2)

The second experiment was performed to assess the uptake and transport of 4 arsenic compounds in plants in the later stages of their growth. In contrast to Experiment 1, the plants were initially grown for the first 4 weeks in solutions without added arsenic. After 4 weeks, the plants were transferred for 7 days to nutrient solutions containing 1 mg L^{-1} of As(III), As(V), DMA and MMA, respectively. The plants were cultivated in 1-L vessels (3 plants per vessel). The experiments for each arsenic form were performed in triplicate. As opposed to Experiment 1, the nutrient solutions were not refilled during the 7-day period. After 7 days, the plants were harvested and the determination of the total arsenic and thiols and the arsenic speciation analysis were performed on the plant tissues. To assess the influence of the plant roots on the stability of the investigated arsenic forms in nutrient solutions, 1 mL of solution was taken from each vessel after 1, 3 and 7 days of cultivation, and the arsenic speciation analysis was performed. Simultaneously, a comparative experiment was carried out. Aerated nutrient solutions, each containing one of the investigated arsenic compounds, were placed for 7 days in vessels without plants. One millilitre of the solution was taken from each vessel after 1, 3 and 7 days, and the arsenic speciation analysis was performed.

2.4. Studies with the exchange of the nutrient medium (Experiment 3)

The results obtained during Experiment 2 indicated that As(III) in the nutrient solution was rapidly oxidised in the presence of plant roots. Because of the instability of this As form, it was difficult to assess the long-term uptake of arsenites by White mustard. After 3 days of cultivation, only arsenates were present in the nutrient solution. Therefore, an additional experiment was carried out. The plants were cultivated for 4 weeks in nutrient solutions without arsenic, as was described in Experiment 2. Then, several plants were transferred for 1 day to a nutrient solution containing 1 mg L^{-1} of As(III). After 1 day, the plants were harvested, and the total determination of arsenic, arsenic speciation analysis and thiol determination was performed on the plant tissues. The remaining plants were transferred for 7 days to a nutrient solution containing 1 mg L^{-1} of As(III). The nutrient solution was removed daily and replaced with a fresh solution containing As(III), in contrast to Experiment 2. After 7 days, the plants were harvested, and the determination of the total arsenic, arsenic speciation analysis and thiol were performed.

2.5. Total arsenic determination

Approximately 250 mg of dried plant material and 3 mL of concentrated HNO_3 were placed in PTFE vessels and digested in a microwave system (Ethos 1 Advanced Microwave Digestion System, Milestone, Italy). A three-stage program with a maximum temperature of 200 °C and a maximum microwave power of 1000 W was used (5 min: 20–90 °C; 10 min: 90–170 °C; 30 min: 170–200 °C). The digested samples were transferred to volumetric flasks and diluted to the volume of 25 mL with milli-Q water. All the samples were digested in triplicate. The measurements were carried out with an ICP-MS (Elan 6100 DRC ICP-MS, Perkin Elmer SCIEX, Canada). The arsenic measurements were compared to standard calibration curves. To validate the digestion and the measurement procedure, two reference materials containing certified amounts of arsenic were analysed: Sea Lettuce *Ulva Lactuca* (CRM No 279) and Oriental Tobacco Leaves (CTA-OTL-1).

2.6. Arsenic speciation analysis

An optimal procedure for arsenic extraction was developed in our previous studies, which indicated that the highest extraction efficiency, reproducibility, and stability of particular arsenic compounds were obtained when dry, homogenised plant material was extracted using water without liquid nitrogen pretreatment [36]. Approximately 250 mg of dried sample was placed in the PP vessel. A volume of 10 mL of Milli-Q water was used as an extractant. Triplicate extractions were carried out for 60 min in the ultrasonic bath, and the extracts were centrifuged at 5000 rpm for 5 min. The applied procedure obtained arsenic extraction efficiencies for White mustard tissues within the following ranges: 60–80% for roots, 55–75% for stems and 60–80% for leaves. The total arsenic concentration in the extracts was measured by ICP-MS. The speciation analysis was carried out using liquid chromatography (Agilent 1200, Agilent, USA) with a quaternary pump and a manual injector (100-μL injection loop) coupled to an Elan 6100 DRC ICP-MS. An anion-exchange column (PRP-X100, 250 × 4.1 mm, particle size of 10 μm) (Hamilton, USA) was used to separate the arsenic compounds (arsenite, arsenate, MMA, and DMA). HPLC analysis was performed using a mobile phase that consisted of 0.01 mol L^{-1} of Na_2HPO_4 (80%) and 0.01 mol L^{-1} of NaH_2PO_4 (20%) buffered at pH~6 and a mobile phase flow rate of 2 mL min^{-1}. The ICP-MS measurements were performed under the following conditions: sweep: 5; replicates: 5; dwell time: 100 ms: ICP