Original Research

Comparison of Platinum, Rhodium, and Palladium Bioaccumulation by *Sinapis alba* and their Influence on Phytochelatin Synthesis in Plant Tissues

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Abstract

Phytochelatins are known to play an essential role in xenobiotic detoxification in some plants. Until now, their synthesis in plants exposed to platinum group elements (PGEs), especially Pd and Rh, has not been directly proven. The influence of PGEs on the natural environment, especially on living organisms, is particularly important in view of increasing emissions of these elements from automotive catalytic converters. This paper describes studies related to the identification of the presence of phytochelatins in tissues of white mustard (*Sinapis alba* L.) exposed to Pt, Rh, and Pd salts. Phytochelatins were identified by high-performance liquid chromatography with two types of detection: fluorescence (FLD) for thiols after derivatization, and electrospray ionization mass spectrometry (ESI MS) for thiols in fresh extracts. Our study confirmed the synthesis of phytochelatins in some plant organs under the influence of PGEs, and illustrated the differences in plant response to stress caused by Pt, Rh, or Pd.

Introduction

The widespread introduction of automobile catalytic converters has increased emissions of platinum group elements (PGEs) into the environment. Thus, PGEs are a matter of concern for environmental chemists interested in contamination of roadside ecosystems [1-5]. The level of PGEs in some places, especially Pd and Pt, exceeds 500 ng g^{-1} [6-8]. Although PGEs are emitted in inert form, once they encounter various soil compounds these elements can be incorporated into bioavailable molecules, as has been observed, especially in the case of Pd [9]. As with other metal ions, PGEs could be taken up by plants, causing dysfunction [10, 11]. Analyses of various plants collected near roadways have shown that Pd exhibits the highest bioavailability, followed by Rh and Pt [3]. The literature suggests that metal ions absorbed from soil through the roots can be bound by sulfur-rich substances [12, 13]. The accumulation process takes place mainly in the vegetative parts of plants and decreases in the following

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order: root>stem>leaves [10-12, 14, 15]. Like Cu and Ni, PGEs are considered to be poorly to moderately bioavailable metals [16]. As a response to physiologically unsuitable ecosystem conditions, plants have developed a number of mechanisms to protect them from stress [17]. Synthesis of phytochelatins (PCs) is an important process controlling the concentration of some metal ions inside the plant cells [18]. These low molecular weight peptides (with the structure of (γ-Glu-Cys) n-Gly, n = 2-11) are able to form complexes with metal ions, and then transport the complexes to the vacuole [19]. The synthesis of PCs in higher plants has been demonstrated under the influence of Cd, Pb, or As [20-22]. Recently the influence of Pt(IV) on the activity of the phytochelatin synthase enzyme, which is involved in the formation of phytochelatins, was examined in maize and pea [23]. The synthesis of phytochelatins has also been confirmed in the tissues of *Arabidopsis thaliana* L and *Sinapis alba* L. under the influence of Pt(II) [10, 24]. So far there is no available information about PCs synthesis in plants under exposure to stress associated with the presence of other PGEs. Nevertheless, some authors have suggested that this mechanism could be worth consideration [25].

The main goal of the study was to prove that PCs are involved in the detoxification process of Pt, Pd, and Rh taken up by *S. alba*, described in the literature as a species with a high xenobiotics bioaccumulation factor [21, 26].

Material and Methods

Cultivation of *Sinapis alba* L.

Sinapis alba L. was cultivated in nutrient solution containing the following: 500 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 150 mg L^{-1} KNO_3, 150 mg L^{-1} $MgSO_4$, 150 mg L^{-1} KH_2PO_4, 0.482 mg L^{-1} $MnSO_4 \cdot 5H_2O$, 0.0185 mg L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 8.0 mg L^{-1} EDTAFeNa, 1.25 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 0.575 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, and 1 mL of 1 mol L^{-1} Tris (hydroxymethyl) aminomethane (all reagents were of analytical grade). 4 L containers (9 seedlings per container) were placed in a growth chamber at 22ºC/20ºC (16h day/8h night) and continuously aerated. White mustard grew for a period of 3 weeks. After one week, nutrient solutions were supplemented with 0.5 mg L^{-1} or 1.0 mg L^{-1} Pt, Pd or Rh chloride, introduced as a 1 g L^{-1} stock solution, and plants (n = 9) were exposed to the presence of xenobiotic for 2 weeks. After 3 weeks plants were harvested and split into leaves, stems, and roots, or roots and aboveground parts. Oven-dried plant material (60ºC) was pulverized in an agate ball mill (FRITSCH, Germany).

Total Elements Content Analysis

Homogenised samples of dried leaves, stems, and roots were digested with concentrated nitric acid in an Ethos 1 Advanced Microwave System (Milestone, Italy) according to the procedure described in the previous paper [26]. In short, 100-250 mg homogenized plant materials were placed in a Teflon vessel and digested for 45 min with 3.0 mL concentrated nitric acid in a close microwave system (5 min 20-90ºC; 10 min 90-170ºC, 30 min 170-200ºC). Each sample was digested at least 3 times. After digestion, samples were diluted with ultrapure water obtained with Milli-Q System (Millipore, USA) to a volume of 25 mL. The measurements of total content of platinum, palladium, and rhodium in plant tissues were carried out with an ICP MS (Nexion 300D, Perkin Elmer, USA) after appropriate sample dilution. During ICP MS three isotopes of Pt (^{194}Pt, ^{195}Pt, ^{196}Pt), three isotopes of Pd (^{105}Pd, ^{106}Pd, ^{108}Pd), and one isotope for ^{103}Rh were controlled. The relative abundance of isotopes was in good agreement with those obtained for standard solutions of Pt and Pd.

Derivatized Thiol Compounds Analysis

In order to verify the presence of phytochelatins in *Sinapis alba* L. tissues, during presented studies two analytical techniques of increasing sophistication were used. As phytochelatins are known to be very unstable, to indicate their presence in plant tissue a derivatization procedure was simultaneously performed. Detailed procedure of thiol analysis was described in our previous work [26]. Shortly, 300 mg of fresh plant material with the addition of 1 mol L^{-1} NaOH (100 μL), 6 mol L^{-1} $NaBH_4$ (100 μL), 6.3 mmol L^{-1} DTPA (1.8 mL), and quartz sand were ground in a mortar. 250 μL of centrifuged solution was mixed with 200 mmol L^{-1} HEPPS buffer (450 μL) and 20 mmol L^{-1} monobromobimane (mBBr) (10 μL). After 30 min of thiol derivatization, 1 mol L^{-1} methanesulphonic acid (MSA) (300 μL) was added to stop the reaction. Analysis by HPLC FLD (Agilent Technologies, USA) was carried out under the conditions optimized and described in previous studies [21]. Filtrated (0.45 μm syringe filter) sample of derivatized thiols was separated on C18 column (Zorbax XDB, 4.6 × 250 mm, Agilent Technologies, USA) with 0.1% trifluoroacetic acid (TFA) and acetonitrile (ACN) in gradient elution program (0-10 min with 8-12% ACN and 10-40 min with 12-35% ACN). Qualitative and quantitative analysis of phytochelatins in plant tissues was made by HPLC FLD method by comparing heights and retention times of signals received for filtrates, to peaks obtained for standard solutions: PC_2 (22.5 min), PC_3 (26.5 min), and PC_4 (29.1 min.).

Underivatized Thiol Compounds Analysis

Extraction of thiols was conducted with 1% formic acid (HCOOH) on triturated in the presence of liquid nitrogen fresh plant material according to a procedure described previously [24]. The samples were shaken for 90 minutes at 1ºC (JWE 357, Poland) and then centrifuged at 6000 rpm at 1ºC for 5 min (5430 R,
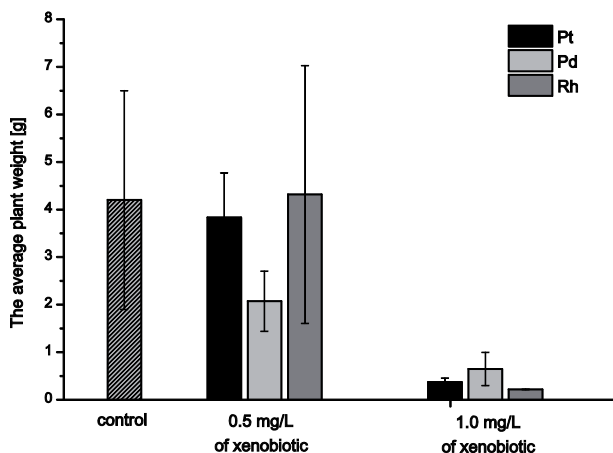
Fig. 1. Average biomass of whole *Sinapis alba* plant (n = 9), hydroponicaly cultivated with the addition of 0.5 mg L^{-1} or 1.0 mg L^{-1} of platinum, palladium, or rhodium salts.
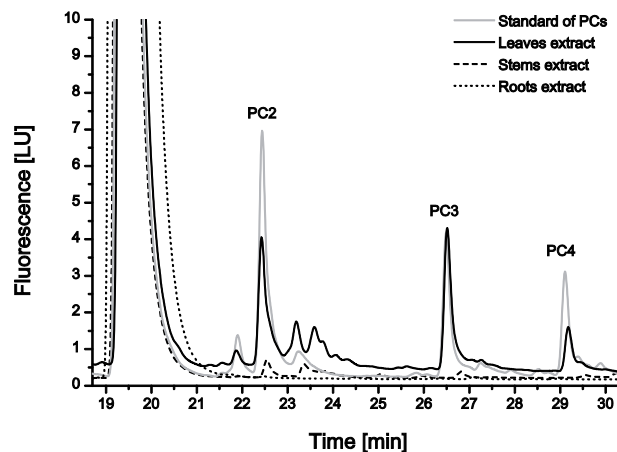

Fig. 2. The HPLC FLD chromatograms of derivatized extract from roots, stems, and leaves of *Sinapis* alba L. exposed to 1.0 mg L^{-1} PdCl$_2$ for 2 weeks, and a derivatized mixture of three phytochelatins (PC$_2$, PC$_3$, and PC$_4$)

Eppendorf, Germany) and filtered (0.45 μm). ESI MS analysis of all plant extracts containing underivatized thiol compounds was completed within 8 hours in a 3200 QTRAP System (SCIEX, USA) connected with a Shimadzu LC System (Shimadzu, Japan). LC-MS/MS analysis started with chromatographic separation on C18 column (Luna, 100 x 2.1 mm, 5 μm, Phenomenex, USA) with a mobile phase consisting of 8 mmol L^{-1} HCOOH and ACN in gradient mode (0-5 min 8% ACN, 5-15 min 8-35% ACN, 15-16 min 35% ACN, 16-18 min 35-80% ACN, 18-21 min 80%, 21-22 min 80-8%). Phytochelatins, obtained in positive-ion mode, were identified based on m/z value by MS and retention times of signals obtained for samples and standard solutions. PC$_2$(mBBr)$_2$ M$^+$ m/z 920 (15.0 min), PC$_3$(mBBr)$_3$ M$^+$ m/z 1342, and M^{2+} m/z 672 (17.0 min); PC$_4$(mBBr)$_4$ M^{2+} m/z 883 (19.0 min) were checked for thiols after derivatization, while in samples without thiols derivatization the following signals were monitored: PC$_2$ m/z 540 (3.4 min), PC$_3$ m/z 772 (6.4 min), and PC$_4$ m/z 1004 (11.8 min) [27].

All graphs were prepared in Origin 8.5 software (OriginLab, USA).

Table 1. Total contents of Pt, Pd, and Rh in *Sinapis alba* tissues (C+SD / μg g^{-1} d.w.; n = 3).

	Pt	Pd	Rh
0.5 mg L^{-1}			
Leaves	1.06±0.02	1.05±0.06	2.55±0.03
Steams	14.9±0.4	51.6±5.0	7.8±0.1
Roots	762±20	663±12	437±59
1 mg L^{-1}			
Aboveground parts	95.8±1.2	30.6±1.4	145±6
Roots	5973±140	1958±40	74.2±1.2

Results and Discussion

Analysis of plants cultivated with 1 mg L^{-1} of PGEs revealed significant biomass reduction in comparison to control plants cultivated without xenobiotics (Fig. 1). The plants exposed to the higher concentration of PGEs suffered stunted growth, deformation, discoloration, and drying of leaves. For crops grown with the lower PGE concentration, considerable biomass reduction was observed only in the case of Pd.

Based on total element concentrations it was found that PGEs were absorbed by the plants' roots and transported to aboveground plant organs (Table 1). Simultaneous analysis of control plants showed Pt, Rh, and Pd concentrations not exceeding 0.1 μg g^{-1} in aboveground organs and 2.0 μg g^{-1} in roots. In PGE-treated cultivation, the highest accumulation factors (AF = $C_{plant}/C_{nutr.sol.}$) were reached for roots (up to 1500 for 0.5 mg L^{-1} and 6000 for 1 mg L^{-1}). Only in the Rh-treated plants (cultivation with 1.0 mg L^{-1}) AF for roots was below 100, two times lower than for aboveground parts, yielding a high translocation factor (TF = C_{shoot}/C_{root} ≈ 2). This phenomenon had already been observed during our studies with thallium [21, 28-30]. For Pt and Pd TF was about 0.015. Among plants cultivated with 0.5 mg L^{-1} PGE, TF were the highest for those treated with Pd, but did not exceed 0.08 for stems. Similar results were obtained when [Pt(NH$_3$)$_4$(NO$_3$)$_2$] was added to the nutrient solution (TF ≈ 0.1) [24].

During these studies our attention was focused on identifying the presence of cysteine-containing phytochelatins in *S. alba* as stress metabolites. This kind of defense mechanism has been already proven in *S. alba* exposed to As [26] and Pt compounds [Pt(NH$_3$)$_4$(NO$_3$)$_2$] [24].

As far as synthesis of phytochelatins is considered, among all PGEs only responses of plants cultivated with Pt addition are described in the literature [24]. Based
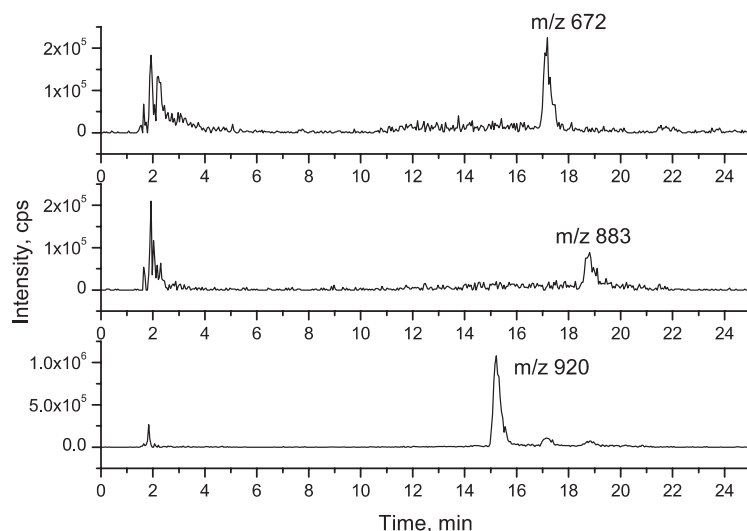
Fig. 3. HPLC ESI MS chromatograms of derivatized leaf extract of *Sinapis* alba L. exposed to 1.0 mg L^{-1} PdCl$_2$ for 2 weeks; peaks with pseudomolecular masses m/z 672, 883, and 920 indicate the occurrence of PC$_3$(mBBr)$_3$, PC$_4$(mBBr)$_4$, and PC$_2$(mBBr)$_2$, respectively.

on the analysis completed by HPLC FLD (Fig. 2) and HPLC ESI MS (Figs 3-4), we found that phytochelatins are synthesized to the greatest extent in plants cultivated in the presence of palladium ions (Fig. 5). Regardless of the Pd concentration in nutrient solution, all three phytochelatins were detected in leaves of *S. alba*. In stems of plants treated with 1.0 mg L^{-1} of Pd, two phytochelatins (PC$_2$ and PC$_3$) were detected, while 0.5 mg L^{-1} caused synthesis of phytochelatin PC$_2$ only. No phytochelatin was observed in plant roots although the Pd concentration in roots was the highest. In leaves,

stems, and roots of plants cultivated in the presence of Pt, independent of xenobiotic concentration in nutrient solution, phytochelatin PC$_2$ was detected, identically to [Pt(NH$_3$)$_4$(NO$_3$)$_2$]-treated plants, as presented previously [24]. The higher Pt concentration (1.0 mg L^{-1}) caused additional synthesis of phytochelatin PC$_3$, but only in plant roots.

In the case of Rh, as for Pd, no phytochelatins were observed in plant roots, independent of Rh concentration in nutrient solution. Phytochelatin PC$_2$ was detected in leaves of *S. alba* from both cultivations. The higher
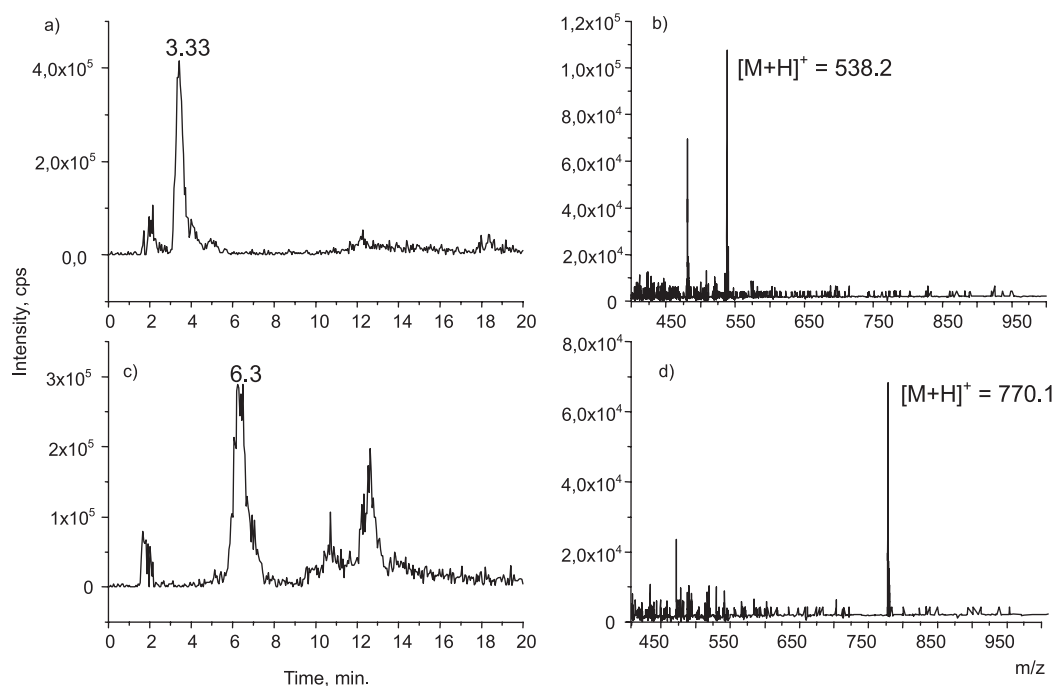

Fig. 4. HPLC ESI MS chromatograms recorded for leaf extract of *Sinapis* alba L. exposed to 1.0 mg L^{-1} PdCl$_2$ for 2 weeks; the peak on retention time 3.33 min (A) with pseudomolecular mass m/z 538.2 (B) indicates the occurrence of free phytochelatin PC$_2$, while the peak with retention time 6.3 min (C) with pseudomolecular mass m/z 770.1 (D) indicates the presence of free PC$_3$.
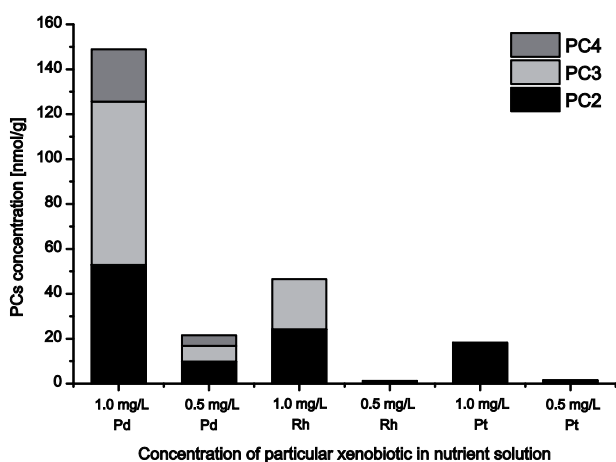
Fig. 5. Concentration of phytochelatins PC_2, PC_3, and PC_4 in leaf extracts of *Sinapis alba* cultivated in nutrient solution with the addition of 1.0 or 0.5 mg L^{-1} Pd, Rh, and Pt salts, respectively.

concentration of Rh caused synthesis of PC_2 and PC_3 in leaf and stem tissues.

Conclusions

Comparing the plant response to all three PGE salts, it should be underscored that they affect *S. alba* to different extents – not only where plant morphology is concerned. The strongest influence of xenobiotics on plant morphology, biomass production, and phytochelatin synthesis was observed in the case of Pd. The presence of phytochelatins in *S. alba* tissues suggests that PGEs are responsible for activation of the phytochelatin synthase enzyme and may be complexed by those metal-binding oligopeptides, which can reduce their potential toxicity.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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