Characterization of lead–phytochelatin complexes by nano-electrospray ionization mass spectrometry

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The role of phytochelatins (PCn) metal-binding oligopeptides with the general structure (γ-Glu-Cys)n-Gly (n = 2–11) in metal detoxification is assumed to be based on immobilization of metals, which prevents binding of metals to important biomolecules. Although induction of phytochelatin synthesis has often been observed in algae upon exposure to metals, direct evidence for binding of the inducing metal to phytochelatins is scarce. In this study, a nano-electrospray ionization mass spectrometry (nano-ESI-MS) method is developed for identification and characterization of Pb(II)–PCn and Zn(II)–PCn complexes. Complexes of Pb(II) with standard PCn (n = 2–4; 0.25 mM Pb(II) and 0.5 mM PCn) were examined by nano-ESI-MS with respect to their stoichiometry. Pb–PCn mass spectra indicated the presence of the [M + H]+ peak of PCn and complexes with various stoichiometries. Analysis of Pb–PC2 allowed the identification of four different complexes observed at m/z 746.10, 952.06, 1265.24, and 1491.20, corresponding to [Pb–PC2]+, [Pb2–PC2]+, [Pb–(PC2)2]+, and [Pb2–(PC2)2]+. Their m/z indicated coordination of Pb(II) by PC2 through the thiol groups of PC cysteine and possibly carboxylic groups. For each of the standard PC3 and PC4, two different complexes were observed, corresponding to Pb–PC3, Pb2–PC3, Pb–PC4, and Pb2–PC4. The measured isotopic patterns were for all complexes identical to the theoretical isotopic addition. Zn(II) (0.125–5 mM) to previously formed Pb–PC2 complexes showed the appearance of the [Zn–PC2]+ complexes at m/z 602.05 and the decrease of the [Pb–PC2]+ peak. These findings corroborate the postulated Pb–PC complexes from a previous study using size exclusion chromatography of PC extracted from algae, as well as the concurrent formation of Pb–, Zn–, and Cu–PC complexes in algae.

Keywords: phytochelatin, mass spectrometry, nano-ESI-MS, lead, thiol

INTRODUCTION

Phytochelatins (PCs) are known to be induced in response to exposure to various metals in plants (Rauser, 1995; Zenk, 1996) and algae (Gekeler et al., 1988; Ahner et al., 1995; Le Faucheur et al., 2005; Scheidegger et al., 2011a). These metal-binding oligopeptides with the general structure (γ-Glu-Cys)n-Gly (n = 2–11) are assumed to bind metals through thiolate coordination and are involved in metal homeostasis and detoxification. The role of PCs in metal detoxification likely results from immobilization of metals, preventing non-specific binding to important biomolecules, followed by the transport of the Me–PC complexes into the vacuole of the algal cell, or its excretion. In our previous studies, induction of phytochelatins by exposure of Chlamydomonas reinhardtii to Pb(II) has been observed (Scheidegger et al., 2011a). Binding of Pb(II) to phytochelatins has been postulated based on separation of metal complexes from C. reinhardtii by size exclusion chromatography (SEG, Scheidegger et al., 2011b). However, direct evidence for binding of the inducing metal to phytochelatins is scarce. It is therefore of interest to attempt to directly characterize metal–phytochelatin complexes.

Several analytical methods such as chromatographic separation (gel filtration or HPLC) coupled with UV detection, flame atomic absorption spectrometry (AAS), radio-active labeling, differential pulse polarography, and inductively coupled plasma mass spectrometry (ICP-MS) have been used to analyze PCn and metal–phytochelatin complexes (Me–PC; Grill et al., 1985; Maitani et al., 1996; Leopold and Günther, 1997; Leopold et al., 1999, 2000; Schmoger et al., 2000; Scarano and Morelli, 2002; Cruz et al., 2003; Kobayashi and Yoshimura, 2006). These methods, however, do not provide exact molecular weight, stoichiometry, or composition of Me–PCn complexes. In most in vivo studies focusing on Me–PC complex characterization, Me–PC complexes were isolated by gel filtration and the resulting eluate fractions were further analyzed for PC and metal content. PC detection often involves acidification and derivatization, which lead to dissociation of the Me–PC complexes, followed by HPLC analysis. Based on the detected molecular weight range obtained from gel filtration and the PC oligomers detected by HPLC, assumptions on stoichiometry and composition of the Me–PC complexes can be made; however, unambiguous characterization of Me–PC complexes regarding stoichiometry and composition is not possible with these methods.

A technique to precisely detect and characterize the Me–PCn complexes is thus required. Several studies reported identification
the stoichiometry of the Pb–PC2–4 complexes is derived.

**MATERIALS AND METHODS**

**CHEMICALS**

Pb(NO3)2, CuSO4, and ZnSO4 salts, ammonium acetate (NH4CH3COO), ammonium carbonate ((NH4)2CO3; pH 7), polylysine, and 3-morpholinopropanesulfonic acid (MOPS) used in this study were analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). Phytochelatin standards (PC2, PC3, and PC4) were obtained from Invitrogen (San Diego, CA, USA). Formic acid was a suprapure chemical obtained from Merck (Darmstadt, Germany). Ultrafree-MC centrifugal filters (0.45 μm cut-off) were ordered from Millipore AG (Zug, Switzerland).

**SAMPLE PREPARATION**

In preliminary experiments the solvent mixture for the analysis of Pb–PC complexes was optimized. The following sample composition resulted in the highest signal intensities in nano-electrospray ionization mass spectrometry (nano-ESI-MS) analysis. The ratio of Pb to Pb was in the range observed in algal cells (Scheidegger et al., 2011a,b). Pb and Pb were mixed, resulting in final concentrations of 0.5 mM Pb and 0.25 mM Pb(NO3)2 in 100 mM NH4CH3COO and 50 mM (NH4)2CO3. Complex formation was allowed, followed by the addition of the Cu or Zn. Sample composition and ESI-MS conditions to analyze Pb complexes with standard phytochelatins (n = 2–4) are optimized. The stoichiometry of the Pb–PC2–4 complexes is derived.

**RESULTS**

The full-scan mass spectrum of the Pb–PC2 complexes (Figure 1) was dominated by the singly charged [M + H]⁺ ion of PC2 at m/z 540.1427, which matches the elemental composition of protonated PC2 (C18H30N5O10S2) of 540.1429 with an error <0.2 ppm and shows the expected isotopic distribution with a mass assignment <0.5 ppm for signals higher than 10% relative abundance (data not shown). In addition to the protonated molecular ion peaks at m/z 536.3 and 538.4 were also present (data not shown), probably corresponding to oxidized PC2. A singly charged PC2 dimer and its oxidation products could be seen at m/z 1079.2783, 1077.2632, and 1075.2474. The four peaks observed at m/z 746.1034, 952.0641, 1285.2379, and 1491.1964 correspond to the molecular weight of singly charged [Pb–PC2]⁺, [Pb2–PC2]⁺, [Pb–(PC2)2]⁺, and [Pb2–(PC2)2]⁺ complexes (Figure 1). Pb–PC2 and Pb2–PC2 were present in sufficient intensity to detect the Pb-specific isotopic pattern of both complexes. The measured isotopic pattern of [Pb–PC2]⁺ in the m/z range 740–752 is shown in Figure 2 (front). The relative intensity of the peaks at m/z 742.11, 744.10, 745.10, and 746.10 was observed in a ratio, which reflects the distribution of Pb isotopes (naturally occurring ratio: 204Pb 1.5; 206Pb 23.6; 207Pb 22.6; 208Pb 52.3%). The measured isotopic pattern matched the theoretical pattern (Figure 2, back), with a mass error of 0.7 ppm or better for signals higher than 10%. Similarly, the isotopic pattern observed for [Pb2–PC2]⁺ at m/z 944–958, including the isotopic pattern of two Pb ions, matched the simulated spectra (Figure 3), with a mass error of 1.1 ppm or less. The signal intensities of the complexes involving one or two Pb ions and two PC2 molecules were too low for isotope pattern detection.
FIGURE 1 | Nano-ESI-MS full-scan spectrum (m/z 300–1,600) of 0.5 mM PC2 (C18H29N5O10S2) and 0.25 mM Pb(NO3)2 in 100 mM NH4CH3COO, 50 mM (NH4)2CO3, and 0.1% HCOOH (pH 6). X = matrix ions.

FIGURE 2 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb–PC2]+ complex m/z 740–752. Isotopic distribution of Pb is identified in the peak distribution at m/z 742.11 (204Pb, 1.5%), 744.10 (206Pb, 23.6%), 745.10 (207Pb, 22.6%), and 746.10 (208Pb, 52.3%).

FIGURE 3 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb2–PC2]+ complex m/z 944–958. Isotopic distribution of two Pb (204Pb 1.5; 206Pb 23.6; 207Pb 22.6; 208Pb 52.3%) is identified in the peak distribution of the m/z 948.06–952.06.

Analysis of Pb–PC3 samples showed the [M + H]+ peak for PC3 at m/z 772.1946 matching the corresponding sum formula (C26H43N7O14S3; data not shown). Similar to PC2, a peak at [M + H–2]+ (m/z 770.1790), not present in the theoretical spectra, was present at high signal intensity. Two peaks at m/z 978.1559 and 1184.1151 corresponding to the molecular weight of singly charged [Pb–PC3]+ (mass error 0.3 ppm) and [Pb2–PC3]+ (mass error 1.2 ppm) were detected. The measured isotopic pattern and the theoretical spectra were almost identical for both detected complexes (Figures 4 and 5).

Analysis of the Pb–PC4 spectra showed the [M + H]+ peak for PC4 at m/z 1004.2406, corresponding to C34H55N9O18S4. Comparison of the [M + H]+ peak at 1004.2406 for PC4 to the theoretical spectra shows an excellent match of the isotopic patterns except for the presence of the [M + H–2]+ peak (m/z 1002.2238) as observed for PC2 and PC3 and the [M + H–4]+ peak (m/z 1000.2084). Two peaks corresponding to the molecular weight of the PC4 complexes, [Pb–PC4]+ and [Pb2–PC4]+, were detected at m/z 1210.1986 (mass error 7.3 ppm) and 1416.1556 (mass error 8.8 ppm). The measured and theoretical isotopic patterns
FIGURE 4 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb–PC₃]⁺ complex m/z 972–984. Isotopic distribution of Pb (²⁰⁴Pb 1.5; ²⁰⁶Pb 23.6; ²⁰⁷Pb 22.6; ²⁰⁸Pb 52.3%) is identified in the peak distribution at m/z 974.15, 976.15, 977.15, and 978.16.

FIGURE 5 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb₂–PC₃]⁺ complex m/z 1,178–1,190. Isotopic distribution of two Pb (²⁰⁴Pb 1.5; ²⁰⁶Pb 23.6; ²⁰⁷Pb 22.6; ²⁰⁸Pb 52.3%) is identified in the peak distribution of the m/z 1,180.11–1,184.12.

FIGURE 6 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb–PC₄]⁺ complex m/z 1,206–1,218. Isotopic distribution of Pb (²⁰⁴Pb 1.5; ²⁰⁶Pb 23.6; ²⁰⁷Pb 22.6; ²⁰⁸Pb 52.3%) is identified in the peak distribution at m/z 1,208.20, 1,209.20, and 1,210.20.

FIGURE 7 | Measured (front) and simulated spectrum (back) of the isotopic pattern of the [Pb₂–PC₄]⁺ complex m/z 1,410–1,422. Isotopic distribution of two Pb (²⁰⁴Pb 1.5; ²⁰⁶Pb 23.6; ²⁰⁷Pb 22.6; ²⁰⁸Pb 52.3%) is identified in the peak distribution of the m/z 1,412.15–1,416.16.

are shown in Figures 6 and 7. In addition, a peak at m/z 605.6035 was observed, matching the isotopic pattern of [Pb–PC₄]²⁺ (data not shown).

COMPETITION BETWEEN Cu OR Zn AND Pb FOR PC₂ BINDING
Addition of Zn to Pb–PC₂ complexes resulted in the appearance of the [Zn–PC₂]⁺ peak at m/z 602.0544 already at the lowest Zn concentration (Figure 8A). In addition, the Pb–PC₂ peak was observed to decrease with increasing Zn concentration (Figure 8). Increasing metal concentration leads to a decrease of all PC signals and to an increase of the ratio between the [M + H]⁺ peak for PC₂ at m/z 540.14 and the [M + H–2]⁺ peak at m/z 538.14. The isotopic pattern of [Zn–PC₂]⁺ matched with the theoretical distribution of Zn (naturally occurring ratio: ⁶⁴Zn 48.6; ⁶⁶Zn 27.9; ⁶⁷Zn 4.1; ⁶⁸Zn 18.8; ⁷⁰Zn 0.6%; Figure 9).

At the highest Zn concentration (5 mM) and at all Cu concentrations no Zn–PC or Cu–PC complexes were detected (data not shown).

DISCUSSION
To test the applicability of nano-ESI-MS for the analysis of Me–PC complexes, complexes of Pb with standard PCₙ (n = 2–4) were analyzed. A method for nano-ESI-MS was developed to characterize in vitro formed Me–PC complexes which might also be used for characterization of in vivo Me–PCₙ complexes.
Considering that the mass spectra of the Pb–PCₙ complexes were dominated by the [M + H]⁺ peak of the corresponding PCₙ, it indicates that either not all PC was involved in complex formation, dissociation of Pb–PC complexes occurs during sample analysis, or that the complexes formed were neutral and therefore not visible in the nano-ESI-MS spectra. Furthermore, the relatively low pH 6, needed for optimal ionization, may lead to some complex dissociation.

The PCₙ seem to occur mainly in charge state 1⁺ with the conditions used, as no signal was detected that corresponds to the doubly charged ion. The isotopic pattern of analyzed PCₙ was completely resolved. For all PCₙ the isotopic distribution matched the theoretical spectra of the corresponding elemental composition and mass accuracy was high (<2 ppm for ions <1,000 Da and with relative intensities higher than 10%). The [M + H - 2]⁺ peaks observed for PC₂–PC₄ as well as the [M + H - 4]⁺ peak observed for PC₄, indicate the formation of one or two intramolecular disulfide bonds between cysteine thiol groups within the PC. The formation of a disulfide bond results in a loss of two hydrogens and therefore a shift from [M + H]⁺ to [M + H - 2]⁺, which was also observed in other studies (Yen et al., 1999; Navaza et al., 2006).

Analysis of Pb–PC complexes revealed the in vitro formation of Pb–PCₙ complexes with various stoichiometries and compositions. The m/z detected for the [Pb–PC₂]⁺ complex allows two different covalent complexes, assuming Pb coordination through thiol groups of PC cysteine. Either Pb is coordinated by one thiol group whereas the other is present as reduced thiol group, or the Pb ion is coordinated by both thiol groups present in PC₂. In the second case, additional protonation of the complex must occur to result in a singly charged complex detectable by nano-ESI-MS. For the [Pb₂–PC₂]⁺ complex the m/z corresponds to [PC₂ + 2Pb - 3]⁺, indicating a loss of three H⁺. This observation suggests that in addition to the two protons from the SH–groups, one proton is lost from a carboxylic group. Further studies would be required to examine whether the complex formation between one Pb and PC₂ involves only thiol groups or Pb is coordinated by one thiol and one carboxylic group. Similarly, another study observed the loss of 2H⁺ and 4H⁺ for the binding of two Cd ions to standard PC₃. Binding of a third Cd ion to PC₃ was not accompanied by the loss of H⁺. The authors suggested the formation of complexes that involve two thiol coordinated cadmium ions and a Cd ion which is bound electrostatically to the Cd₂–PC₃ complex (Yen et al., 1999). To investigate whether the coordination of metals by PC is dependent on the metal and/or the chain length of PC needs to be further investigated.

From mass considerations four Pb–PC₂, two Pb–PC₃, and two Pb–PC₄ complexes were identified. To prove that both Pb and PCₙ are contributing to the detected signals, the measured isotopic pattern was compared to the theoretical isotopic pattern.
The isotopic patterns of the Pb–PC complexes are complex owing to the PC complexes. In a voltammetric study with multivariate curve resolution methods (Alberich et al., 2008), in addition, accurate mass measurement of Pb ions in the presence of Cu(II), potential metal-binding sites. This could also explain the signal loss measured and theoretical isotopic pattern proves the presence of the two Pb ions (Figure 3). Formation of [Pb–PC2] and [Pb–PC2] complexes was also observed using differential pulse polargraphy (Alberich et al., 2007). Similar observations were made for Pb–PC3 and Pb–PC4 complexes, showing a good match between measured and theoretical patterns and a loss of 2H for each additional bound Pb ion. Surprisingly no complexes including three and four Pb ions were observed for PC3 and PC4. These results are also in agreement with the complexes observed using voltammetric methods (Alberich et al., 2008). In addition, accurate mass measurements confirmed the proposed elemental compositions.

The appearance of the [Zn–PC2]+ peak in the presence of Zn is indicative of complex formation between PC and Zn, which was confirmed by the isotopic pattern for Zn clearly visible in the zoom spectrum (Figure 9). This competition between Pb and Zn may be expected if their complex stability with PC2 is similar to the stability of their complexes with glutathione, for which somewhat higher stability constants for Pb than for Zn are reported (Martell and Smith, 1989). Binding of Zn by PC2 has been shown in a voltammetric study with multivariate curve resolution, and by PC4 using voltammetry and ESI-MS (Cruz et al., 2005; Chekmeneva et al., 2007). The expected increase of the [Zn–PC2]+ peak with increasing Zn concentration was not observed, maybe due to an increase of oxidized PC2 indicated by the increase of the ratio between m/z 538.14 and 540.14, leading to a loss of potential metal-binding sites. This could also explain the signal loss observed with increasing Zn concentration. In presence of Cu(II), phytochelatin oxidation may also explain why no Cu–PC complexes were detected. Similar observations were done in a study with Cd where a signal loss was observed at concentrations higher than 0.3 mM Cd (Yen et al., 1999).

In a previous study using SEC, the presence of PC complexes with Cu, Zn, and Pb was postulated upon analysis of PC from C. reinhardtii exposed to Pb (Scheidegger et al., 2011b). After extraction of the algal cells under native conditions to preserve the metal complexes, PC2 and PC3 complexes were detected in a molecular weight range between 700 and 5,300 Da. PC2 was mainly observed between 1,000 and 1,600 Da and complexes with Me1–2–(PC2)2 were suggested, with [PC2+(PC2)] and [PC2+(PC2)] as the probable most abundant Pb species. The results obtained here are in qualitative agreement with this study, as the formation of [Pb–PC2]+, [Pb–PC2]+, [Pb–(PC2)2]+, and [Pb–(PC2)2]+ is shown. [Pb–PC2]+ and [Pb2–PC2]+ would appear in the SEC fraction 700–1,050 Da, where PC2 and Pb were also detected. The abundance distribution of these complexes obtained by ESI-MS appears to differ somewhat from the SEC results, but it must be taken into account that the ratio of PC–SH to Pb, as well as the pH were different in these two studies. Furthermore, it must be considered that this ESI-MS, albeit a soft ionization technique, may result in dissociation of complexes. The formation of the [Zn–PC2]+ complex after Zn addition also corroborates the results from SEC, which showed the presence of Zn and Cu, as well as Pb, in the PC containing fractions. These results also clearly indicate a possible competition of Zn and Pb for binding to phytochelatins. These findings support the hypothesis that upon induction of PC2 by Pb in algae, the PCn may also be bound to other metals.

The application of nano-ESI-MS to examine Me–PC complexes in algae is challenged by practical issues related to the low intracellular concentration of the Me–PC complexes. Therefore, further research is needed to improve the sensitivity for Me–PC complexes by nano-ESI-MS, or respectively to improve sample preparation to obtain a sufficient amount of PCn from the algae. For example, considering the measured concentration of 30 attomol/cell PC2 in C. reinhardtii cells (Scheidegger et al., 2011a), about 1 L of algal suspension (with a cell density of 8.4 × 105 cell/mL) should be preconcentrated into a small volume (<1 mL) to obtain a sufficiently high PC2 concentration for ESI-MS measurements. In addition, differences between in vivo and in vitro formed Me–PC complexes should be further examined to investigate which factors are determining the distribution among the various complexes.

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REFERENCES

Ahner, B. A., Kong, S., and Morel, F. M. M. (1995). Phytochelatin production in marine algae. I. An interspecies comparison. Limnol. Oceanogr., 40, 649–657.
Alberich, A., Arino, C., Diaz-Cruz, J. M., and Esteban, M. (2007). Soft modelling for the resolution of highly overlapped voltammetric peaks: application to some Pb phytochelatin systems. Talanta 71, 344–352.
Alberich, A., Diaz-Cruz, J. M., Arino, C., and Esteban, M. (2008). Combined use of the potential shift correction and the simultaneous treatment of spectroscopic and electrochemical data by multivariate curve resolution: analysis of a Pb(II)-phytochelatin system. Analyst 133, 470–477.
Bluemlein, K., Raab, A., and Feldmann, J. (2009). Stability of arsenic peptides in plant extracts: off-line versus on-line parallel elemental and molecular mass spectrometric detection for liquid chromatographic separation. Anal. Bioanal. Chem. 393, 357–366.
Bluemlein, K., Raab, A., Meharg, A., Charnock, I., and Feldmann, J. (2008). Can we trust mass spectrometry for determination of arsenic peptides in plants? comparison of LC–ICP-MS and LC–EN-MS/ICP-MS with XANES/EXAFS in analysis of Thurebergia alata. Anal. Bioanal. Chem. 390, 1739–1751.
Chekmeneva, E., Diaz-Cruz, M. J., Arino, C., and Esteban, M. (2007). Binding of Cd2+ and Zn2+ by the phytochelatins (γ-Glu-Cys)4-Gly: a voltammetric study assisted by multivariate curve resolution and electrospray ionization mass spectrometry. Electroanalysis 19, 310–317.
Chen, L., Guo, Y., Yang, L., and Wang, Q. (2007). SEC-ICP-MS and ESI-MS/MS for analyzing in vitro and in vivo Cd-phytochelatin complexes in a Cd-hyperaccumulator Brassica chinensis. J. Anal. At. Spectrom. 22, 1403–1408.
Cruz, B. H., Diaz-Cruz, J. M., Arino, C., and Esteban, M. (2005). Complexation of heavy metals by phytochelatins: voltammetric study of the binding of Cd2+ and Zn2+ ions by the phytochelatin (γ-Glu-Cys)3Gly assisted by multivariate curve resolution. Environ. Sci. Technol. 39, 778–786.
Gekeler, W., Grill, E., Winnacker, E.-L., and Zenk, M. H. (1986). Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch. Microbiol. 150, 197–202.

Grill, E., Winnacker, E.-L., and Zenk, M. H. (1985). Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230, 674–676.

Kobayashi, R., and Yoshimura, E. (1985). Phytochelatin-induction, cadmium accumulation and algal sensitivity to free cadmium ions in Scenedesmus vasculatus. Environ. Toxicol. Chem. 1425–1430.

Leopold, L., and Günther, D. (1997). Investigation of the binding properties of heavy-metal-peptide complexes in plant cell cultures using HPLC-ICP-MS. Fresenius J. Anal. Chem. 359, 364–370.

Leopold, L., Günther, D., Schmidt, J., and Neumann, D. (1999). Phytochelatins and heavy metal tolerance. Phytochemistry 50, 1233–1238.

Maitani, T., Kubota, H., Sato, K., and Yamada, T. (1996). The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of Raudia tincturam. Plant Physiol. 110, 1145–1150.

Martell, A. E., and Smith, R. M. (1989). Critical Stability Constants. New York: Plenum Press.

Navaa, A., Montes-Bayón, M., Leduc, D. L., Terry, N., and Sanz-Medel, A. (2006). Study of phytochelatins and other related thiols as complexing biomolecules of As and Cd in wild type and genetically modified Brassica juncea plants. J. Mass Spectrom. 41, 323–331.

Raab, A., Schat, H., Meharg, A. A., and Feldmann, J. (2005). Uptake, translocation and transformation of arsenate and arsenite in sunflower Helianthus annuus: formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. New Phytol. 168, 551–558.

Rauser, W. E. (1995). Phytochelatins and related peptides – structure, biosynthesis, and function. Plant Physiol. 109, 1141–1149.

Scarano, G., and Morelli, E. (2002). Characterization of cadmium- and lead-phytochelatin complexes formed in a marine microalg. Biometals 15, 145–151.

Scheidegger, C., Behra, R., and Sigg, L. (2011a). Phytochelatin formation and its desglycyl peptide) induced by various metals in root cultures of Raudia tincturam. Plant Physiol. 110, 1145–1150.

Scheidegger, C., Sigg, L., and Behra. (R. 2011b). Characterization of lead induced metal-phytochelatin complexes in Chlamydomonas reinhardtii. Environ. Toxicol. Chem. 30, 2546–2552.

Schmoger, M. E. V., Oven, M., and Grill, E. (2000). Detoxification of arsenic by phytochelatins in plants. Plant Physiol. 122, 793–802.

Vacchina, V., Chassaigne, H., Oven, M., Zenk, M. H., and Lobinski, R. (1999). Characterisation and determination of phytochelatins in plant extracts by electrospray tandem mass spectrometry. Analyst 124, 1425–1430.

Vacchina, V., Lobinski, R., Oven, M., and Zenk, M. H. (2000). Signal identification in size-exclusion HPLC-ICP-MS chromatograms of plant extracts by electrospray tandem mass spectrometry (ES MS/MS). J. Anal. At. Spectrom. 15, 529–534.

Yen, T.-Y., Villa, J. A., and Dewitt, J. G. (1999). Analysis of phytochelatincadmium complexes from plant tissue culture using nano-electrospray ionization tandem mass spectrometry and capillary liquid chromatography/electrospray ionization tandem mass spectrometry. J. Mass Spectrom. 34, 930–941.

Zenk, M. H. (1996). Heavy metal detoxification in higher plants – a review. Gene 179, 21–30.

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