Survey of the Plant Kingdom for the Ability to Bind Heavy Metals through Phytochelatins

Walter Gekeler**, Erwin Grill, Ernst-Ludwig Winnacker*, and Meinhart H. Zenk

Lehrstuhl für Pharmazeutische Biologie der Universität München, Karlstraße 29, D-8000 München 2, Bundesrepublik Deutschland

* Laboratorium für Molekulare Biologie – Genzentrum der Ludwig-Maximilians-Universität, Am Klopferspitz, D-8033 Martinsried, Bundesrepublik Deutschland

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Dedicated to Professor Achim Trebst on the occasion of his 60th birthday

Phytochelatins, Glutathione, homo-Glutathione, homo-Phytochelatins, Heavy-Metal Binding Peptides, Cell Cultures

Differentiated plants and suspension cultures of the taxonomic divisions Bryophyta, Pteridophyta, and Spermatophyta have been investigated as to their ability to detoxify heavy metals like Cd^{2+} through the formation of $(\gamma\text{-Glu}-\text{Cys})_n-\text{Gly}$ peptides, the phytochelatins. Over 200 individual plants have been checked and there was not a single exception observed. Only in the order Fabales several species mainly of the tribus Fabaceae form upon exposure to Cd^{2+} ions peptides of the general structure $(\gamma\text{-Glu}-\text{Cys})_n-\beta\text{-Ala}$, the homo-phytochelatins. The existence of glutathione and homo-glutathione within a given species determines whether phytochelatins or their homo-derivatives are formed. The ability to form phytochelatins for metal homeostasis and metal detoxification is a principal feature of plant metabolism.

Introduction

All living cells are confronted with the dilemma that on one side they need certain amounts of free heavy metal ions (such as Zn^{2+}, Cu^{2+}, Ni^{2+}, etc.) for their normal metabolic function, and on the other side they have to protect themselves from an intracellular excess of these metal ions which would lead to cell death. This dilemma can only be overcome by a stringent regulation of free metal ion concentration within the cells, which can be regulated in several ways such as: metal-binding to cell walls, reduced transport across cell membrane, active efflux, compartmentalization and chelation [1]. The mechanism which has been studied most closely in recent years is chelation. Heavy metals in vertebrates and certain fungi are detoxified by sulfur-rich, 6.5 kDa proteins devoid of aromatic amino acids, the metallothioneins [2]. Plant cells, on the other side, after heavy metal exposure, synthesize small, sulfur-rich peptides that bind heavy metals. They are composed only of 3 amino acids, namely L-cysteine, L-glutamic acid and glycine. Glutamic acid is linked to each cysteine by a γ-peptide linkage. They are not primary gene products. The general structure of this set of peptides is $(\gamma\text{-Glu}-\text{Cys})_n-\text{Gly}$ ($n = 2-11$). They are called phytochelatins (PC's) [1, 3]. In a few members of the Fabales (Leguminosae), phytochelatins are substituted by a peptide family containing a β-alanine carboxy terminus instead of the glycine. These peptides were termed homo-phytochelatins $(\gamma\text{-Glu}-\text{Cys})_n-\beta\text{-Ala}$ ($n = 2-7$) [4]. Phytochelatin induction has been found to occur in some fungi [5, 6], in algae [7] and in some differentiated as well as tissue-cultured higher plants [1]. There remained, however, always the question as to the ubiquity of this metal-binding process in the plant kingdom. Therefore, we undertook the task to screen a large number of plant species belonging to the divisions Bryophyta, Pteridophyta and Spermatophyta.

Abbreviations: GSH, glutathione; h-GSH, homo-glutathione; PC_n, phytochelatin with n-(γ-Glu–Cys) units; h-PC_n, homo-phytochelatin with n-(γ-Glu–Cys) units; dwt, dry weight.

** Present address: Lever GmbH, Rhenanicastraße 78–102, D-6800 Mannheim 81, Bundesrepublik Deutschland.

Reprint requests to Prof. M. H. Zenk.

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Materials and Methods

Biological material

Plant cell cultures were provided by our cell culture laboratory. If not otherwise stated, the cultures were grown at 23 ± 1.5 °C at 70% humidity, 600 lux incandescent light and 100 rpm on gyratory shakers. The media used were: LS = Linsmaier and Skoog

[8]; 4X = Gamborg *et al.* medium [9] containing as hormones 0.5 mg/l indoleacetic acid, 0.5 mg/l naphthaleneacetic acid, 2 mg/l 2,4-dichlorophenoxyacetic acid, 0.2 mg/l kinetin; DAX = Gamborg *et al.* medium [9] containing as hormone 2 mg/l 2,4-dichlorophenoxyacetic acid; MS = Murashige and Skoog [10].

Seeds of plants were obtained from the botanical gardens of Cologne, Bayreuth and Munich. Seedlings were placed on styrofoam rafts so that their roots were exposed to Hoagland-nutrient solution [11] which was aerated (0.5 l air $\times h^{-1} \times l^{-1}$-nutrient medium) at 27 °C at 1200 lux. Exposure to $Cd(NO_3)_2$ was done for 4–12 days at a concentration of usually 20–50 μM. The moss *Marchantia polymorpha* was cultivated under sterile conditions in Knop solution [12] solidified with 1.2% agar. Differentiated plants of several Pteridophyta were exposed to heavy metals in the same way, however, the Hoagland-nutrient solution [11] was diluted to 1/10 strength.

Analytical procedures

Heavy metals were determined by atomic absorption spectroscopy (Perkin-Elmer PE-1100B) with flame mode. Samples (50 mg) were digested with concentrated H_2SO_4 or HNO_3 (50 μl) and analyzed after appropriate dilution (to 1.5 ml). Protein was determined according to [13], SH-groups according to [14]. Phytochelatins were assayed as follows [7, 15]: Tissue was frozen with liquid nitrogen, ground, and to 400 mg powder was added 0.4 ml 1 N NaOH containing 0.4 mg $NaBH_4$. The sample was sonicated (Branson, 3 × 5 sec, setting 4) and subsequently cell debris centrifuged off. The supernatant was transferred into an Eppendorf vial, acidified with 100 μl 3.6 N HCl and put on ice for 15 min. Precipitated protein was removed by centrifugation. 20–250 μl of this clear supernatant were injected into an HPLC system (Spectra-Physics, as described in [7]). Nucleosil (10 C-18) or LiChrosorb (RP-18; 7 μ) 4 × 250 mm columns were used for separation of the peptides. Elution was achieved by a gradient using 0.05% H_3PO_4 in 0–20% acetonitrile–H_2O. Detection was for pure phytochelatins at 220 nm, crude extracts were analyzed by -SH specific detection using Elman's reagent (DTNB) [14]. Post-column derivatization was accomplished by mixing to the eluate of the separation column DTNB reagent (75 μM DTNB in 50 mM $K^{-2}PO_4$ buffer pH 8) at a rate of 2 ml $\times$ min^{-1}. The mixture was passed through a reaction loop (5 ml volume) which corresponds to a reaction time of 1.25 min and subsequently the absorption at 410 nm was recorded and the peak area automatically integrated [15]. Glutathione was used as standard. The nmol peptides were related to dry weight of the plant sample or protein content. One μg of phytochelatin complex could still be quantitated and resolved into individual PC species by this method. This method yielded at least 90% of cadmium PC present in the sample.

Results

The heavy-metal binding peptides from lower plants

It has previously been shown that algae are capable of forming phytochelatins in response to heavy metal stress and that in contrast to previous reports not proteins are responsible for this detoxification but rather the peptides of the $(\gamma\text{-Glu-Cys})_n$–Gly type [7]. In order to extend our knowledge about the ubiquity of the heavy-metal binding phytochelatin system in the plant kingdom, plants of the division Bryophyta and Pteridophyta were tested. As shown in Table I, a member of the mosses, *Marchantia polymorpha,* when grown in axenic culture was capable of forming PC_2 and PC_3. The HPLC profile is given in Fig. 1A. No PC synthesis was detected in the control sample. In the division of Pteridophyta, however, where particularly slow growing plants are found, none of the plants grown in the field or under greenhouse conditions yielded PC synthesis upon exposure to Cd^{2+} in their native soil, except the water fern *Azolla filiculoides,* which could be directly transferred to the heavy metal solution. There was the possibility that the heavy metal was adsorbed to soil particles and could not reach the roots. The availability of heavy metals was achieved by saturation of the soil particles with Al^{3+} first, as described by Fischer [16]. Plants growing in their natural environment, treated with a mixture of 1 mM Al^{3+}, 1 mM Zn^{2+} and 100 μM Cd^{2+} in aqueous solution, reacted clearly by PC induction after a period of 4 days (Table I). Ms. B. Rittgen in our laboratory succeeded also to establish a cell culture of *Equisetum giganteum,* the first cell culture reported for a member of the Equisetales. It could be shown that these suspension cultures reacted immediately and to the same extent as cultures of higher plants towards exposure to 100 μM Cd^{2+} with the synthesis of PC's

Table I. Phytochelatin synthesis in some members of *Sporophyta*.

Species	Order	PC_2[a]	PC_3	PC_4	PC_5	PC_6	Total PC[b]
		[nmol/mg dwt]					
Marchantia polymorpha	Marchantiales	0.5	0.2	0.0	0.0	0.0	1.7
*Selaginella viticulosa**	Selaginellales	0.2	0.1	0.1	0.0	0.0	0.9
*Lycopodium clavatum**	Lycopodiales	0.2	0.0	0.0	0.0	0.0	0.5
*Equisetum giganteum**	Equisetales	0.0	0.5	0.2	0.0	0.0	2.5
Equisetum giganteum S	Equisetales	0.5	0.2	0.2	0.1	0.1	3.3
Azolla filiculoides	Hydropteridales	0.2	0.1	0.0	0.0	0.0	0.7

* Plants in natural substrate irrigated with 1 mM Al^{3+}, 1 mM Zn^{2+} and 100 μM Cd^{2+}.
S: Suspension cultured tissue growing in LS medium challenged with 100 μM Cd^{2+}.
[a]: Corresponds to number of (γ-Glu–Cys) residues in phytochelatin.
[b]: Total amount of (γ-Glu–Cys) residues induced. No phytochelatin peptides were detected in control cultures not exposed to Cd^{2+}.

(Fig. 1B). For induction of PC synthesis in *E. giganteum* on the natural substrate by Cd^{2+}, again masking of the soil particles with Al^{3+} was necessary. The results compiled in Table I demonstrate for the first time that members of the Bryophyta and Pteridophyta are capable of detoxifying excess concentrations of heavy metals by chelation to PC's. Probably, under normal conditions, these plants use the PC system for the homeostasis of heavy metals in their metabolism as was shown for Spermatophyta [17].

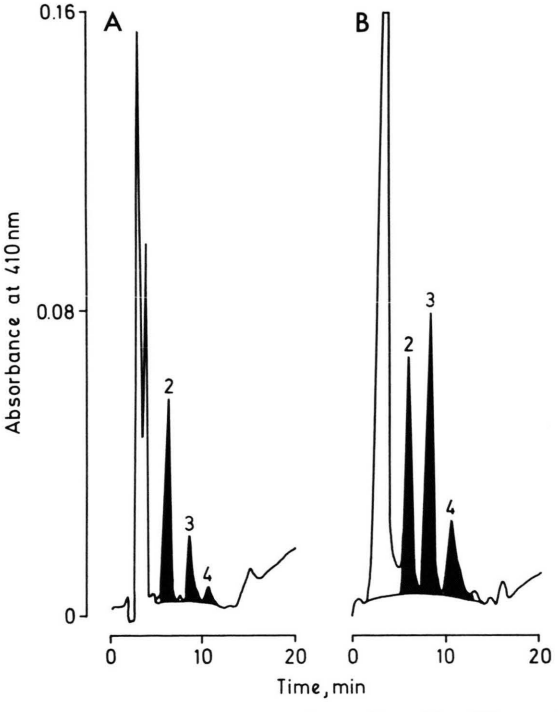

Fig. 1. Reversed phase HPLC profile with -SH specific detection of crude extracts from: A. axenic culture of differentiated plants of *Marchantia polymorpha* exposed to 100 μM Zn^{2+} for 4 days, B. suspension culture of *Equisetum giganteum* exposed to 100 μM Cd^{2+} for 2 days. The shaded peaks represent phytochelatin peptides indexed with the number of (γ-Glu–Cys) residues contained therein. No phytochelatins were detected in crude extracts from untreated controls.

Phytochelatins in Coniferophytina

In the division Spermatophyta, the subdivision Coniferophytina (Gymnospermae) comprises about 800 known species. Some of these species have recently received special attention due to damages reported which are supposed to be of anthropogenic origin and heavy metal pollution was considered to be one possibility [18]. It was therefore of interest to investigate the potential of this group of plants to produce phytochelatins. Both suspension cultures and 1 year old rooted plants (seedlings) were used for these experiments. As shown in Table II, all of the members of the Gymnospermae tested (members of two of the three existing classes) had the ability to form PC's in varying chain length after exposure to Cd^{2+}. All unexposed control plants did not yield evidence for the presence of PC's. The HPLC profiles of cell cultures of *Pinus pinea* (A) and *Gingko biloba* (B) treated with 100 μM Cd^{2+} are shown in Fig. 2 and demonstrate clearly PC formation. We therefore conclude that also all members of the Coniferophytina do chelate heavy metals through the phytochelatin system, involving metal-thiolate complexes.

Phytochelatin induction in Angiospermae

The Angiospermae (Magnoliophytina) are by far the largest subdivision in the plant kingdom, comprising about 250,000 species. In the past we were

Table II. Phytochelatin synthesis in some members of the Gymnospermae.

Species	Order	*	+	PC_2[a] [nmol/mg	PC_3 dwt]	PC_4	PC_5	Total PC[b]
Cycas revoluta	Cycadatae	D	H	4.5	3.0	0.6	0.3	22.1
Gingko biloba	Gingkoatae	S	DAX	1.3	1.0	1.1	0.9	14.8
Abies alba	Pinales	D	H	3.8	2.0	0.0	0.0	13.7
Abies grandis	Pinales	S	4x	0.9	0.2	0.0	0.0	2.3
Picea abies	Pinales	D	H	1.5	0.1	0.0	0.0	3.4
Pinus pinea	Pinales	S	4x	6.8	1.2	0.2	0.0	18.0
Pinus sylvestris	Pinales	D	H	2.5	0.5	0.3	0.0	7.6

[a, b]: Indices as in Table I.
* D = differentiated plant/seedling or S = suspension culture.
+ Media: H = Hoagland-nutrient solution, DAX = Gamborg's *et al.* medium [9] modified with 10^{-5} M 2,4-D and 0.5% NZ-amines, and 4x as described in Materials and Methods.

able to demonstrate that several plant species grown in cell culture when supplied with sublethal concentrations of heavy metals such as Cd^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Ag^{1+}, Hg^{2+} induce PC synthesis, yielding the corresponding PC metal complexes [3, 15]. In order to prove that this detoxification mechanism is of ubiquitous occurrence in Angiospermae we first exposed differentiated plants or cell cultures of 90 species of 85 genera of 48 orders of the Dicotyledoneae class of the angiosperms to Cd^{2+} ions and observed the induction of PC formation. The results of this survey are shown in the abbreviated Table III. Only the 10 most active species, differentiated plants and cell cultures are shown. The list of the species not shown here is available upon request from the corresponding author. For the species which produces the largest number of PC chains found during this survey the HPLC profiles is shown in Fig. 3A. It is *Rauwolfia serpentina* for which peptides up to PC_{11} (23 amino acids) were shown to occur. Retention times of peptides (T_R) during HPLC chromatography do not increase linearly but logarithmically as expressed by the formula given by Sasagawa *et al.* [19]. Application and transformation of his equation gives in the case of phytochelatins:

$$T_{RPCn} - T_{RGSH} = a \times \log n \; (n = 1, 2, 3, 4 \ldots)$$

Retention times for the sequenced PC monomers PC_2–PC_6 (T_{RPCn}) were taken from the chromatogram shown in Fig. 3A (linear acetonitrile gradient $1\% \times min^{-1}$). The retention time for GSH was determined to be 2.38 min. In Fig. 3B the differences in retention time between individual PC members and glutathione ($R_{RPCn} - T_{RGSH}$) were plotted against the log of (γ-Glu–Cys) units. A straight line resulted, with a slope which could be calculated as a = 18.47 min. The transformed equation is therefore:

$$T_{RPCn} = 18.47 \text{ min} \times \log n + 2.38 \text{ min}$$

where n is the number of (γ-Glu–Cys) units in a given phytochelatin molecule. Employing this equation, the retention times of an unknown PC member

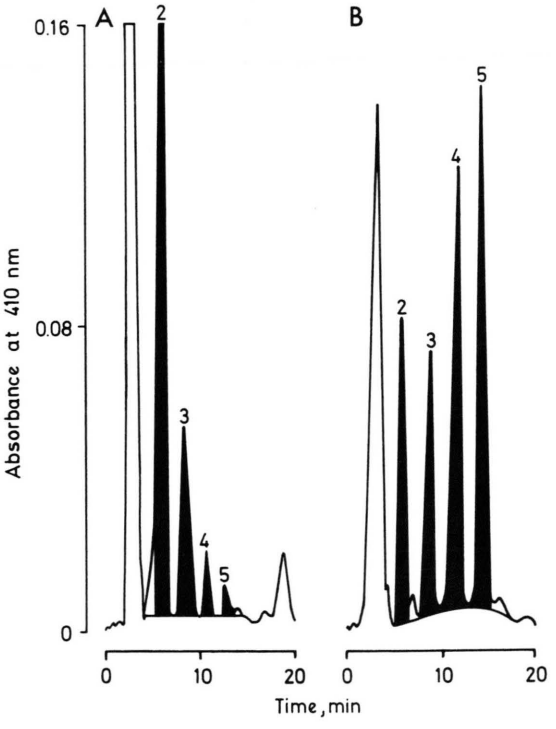

Fig. 2. Reversed phase HPLC profile with -SH specific detection of crude extracts from plants of the subdivision Gymnospermae exposed to 100 μM Cd^{2+} for 3 days: A. suspension cells of *Pinus pinea*, B. suspension cells of *Gingko biloba*. Diagram explanation see legend to Fig. 1.

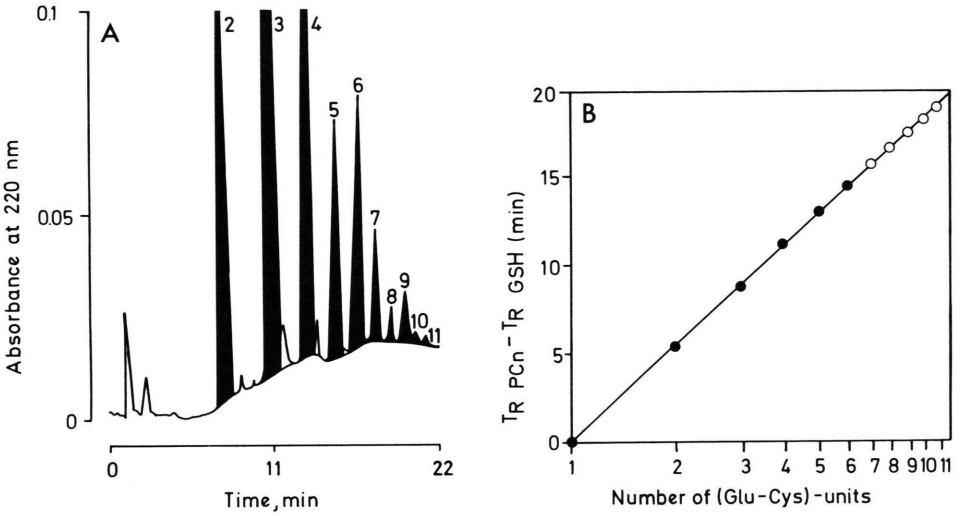

Fig. 3. A. Reversed phase HPLC profile with -SH specific detection of a crude extract of the dicotyledonous angiosperm *Rauwolfia serpentina* exposed to 300 μM Cd^{2+} for 4 days, showing the highest number of phytochelatin molecular species ever found in a plant up to now. B. Retention time (T_R) during HPLC analysis of phytochelatin peptides as a function of the number of (γ-Glu−Cys) units. In the graph, the retention time of PC peptides minus that of glutathione was plotted against the number of (γ-Glu−Cys) units. Closed symbols, values of sequenced peptides PC_{2-6}; open symbols, T_R of peptides PC_{7-11} as taken from Fig. 3A.

Table III. Phytochelatin synthesis in members of the Dicotyledoneae; list abbreviated from a survey of 90 species.

Species	Order	*	+	PC_2[a]	PC_3	PC_4	PC_5	PC_6	Total PC[b]
				[nmol/mg dwt]					
Anthemis arvensis	Asterales	D	H	0.18	0.65	1.10	0.05	0.00	6.95
Sinapis alba	Capparales	D	H	0.49	0.73	0.63	0.20	0.00	6.70
Linum usitatissimum	Geraniales	D	H	0.00	1.78	0.46	1.03	0.38	14.68
Laurus nobilis	Laurales	D	H	0.71	0.30	0.55	0.58	0.12	8.75
Cannabis sativa	Urticales	D	H	2.18	3.16	0.60	0.01	0.00	16.29
Minuartia verna	Caryophyllales	S	LS	0.59	0.57	0.06	0.03	0.00	3.27
Eucommia ulmoides	Eucommiales	S	4x	2.12	0.36	0.14	0.00	0.00	5.87
Linum usitatissimum	Geraniales	S	LS	0.06	0.14	0.18	0.01	0.01	1.40
Capsicum annuum	Solanales	S	DAX	0.17	1.51	0.94	0.30	0.07	10.56
Viola calaminaria	Violales	S	LS	0.94	1.55	0.45	0.14	0.00	9.03

[a, b, *]: Indices as in Table II.
[+] Media as in Table II and in addition: LS = Linsmaier and Skoog [8] medium.

can be calculated. The retention time for the higher PC homologues PC_{7-11} were calculated using this formula and matched perfectly the values found in Fig. 3A. The error in retention times determined for each individual PC member was less than 1%.

In the hope to find plant species which would yield cell cultures having a higher than normal ability to synthesize PC's, *Linum usitatissimum, Gossypium herbaceum, Ipomea purpurea,* and *Tropaeolum majus* cell cultures were induced from seeds. The resulting suspension cultures, however, showed no increased PC synthesis potential when compared to our standard organisms *Rauwolfia serpentina* (Apocynaceae) and *Silene cucubalus* (Caryophyllaceae). Quantitative data are shown for *Linum* (differentiated plant *vs.* cell culture) in Table III. It is also of interest to note that some members of genera in which heavy-metal tolerant plants are found (Violales, Caryophyllales) reacted with an average rate of synthesis of PC's to exposure to Cd^{2+}. PC synthesis

was found also to occur in *Arabidopsis thaliana* and in several species of *Oenothera*. It would be interesting to analyze the genetical basis of PC synthesis using these genetically well characterized organisms.

The same type of survey was done also in the second class of the Angiospermae, the monocotyledoneous plants (Liliatae). As shown in the abbreviated Table IV, again all species tested (23 species of 14 orders) of this class show a response to Cd^{2+} exposure by phytochelatin formation. Some members of the most important food plants: oat, barley, rye, rice, wheat, and maize all produce PC's when challenged with heavy metals. The heavy metal-phytochelatin complexes may therefore be relevant for animal and human nutritional studies since these complexes reflect the state of heavy metal chelation within these plants. HPLC profiles of two species of the class Monocotyledoneae are depicted in Fig. 4 (A: *Avena sativa* (Poales), and B: *Phalaenopsis amabilis* (Orchidales)).

Up to this point all investigated higher and lower plant species without exception produced PC's of the general formula $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ upon exposure to Cd^{2+}.

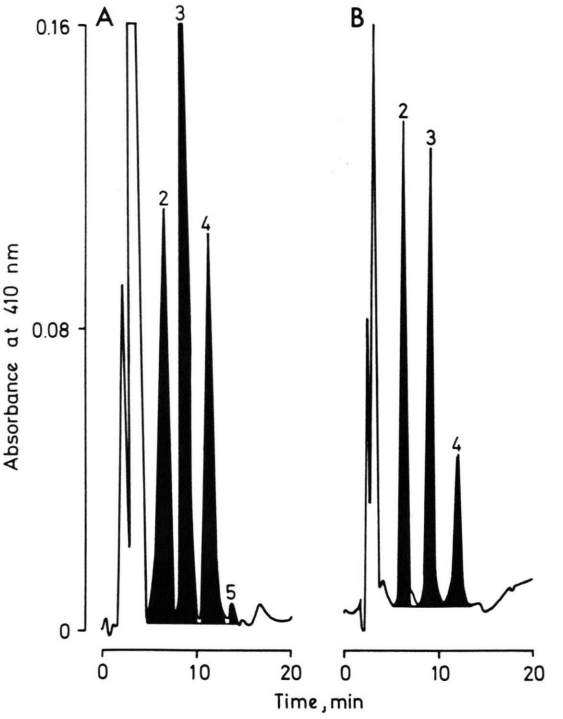

Fig. 4. Reversed phase HPLC profile with -SH specific detection of crude extracts from differentiated seedlings of monocotyledonous plants exposed to 20 μM Cd^{2+} in Hoagland-nutrient solution for 3 days. A. *Avena sativa*, B. *Phalaenopsis hybrida*. Diagram explanation see legend to Fig. 1.

The exception: homo-phytochelatins in the Fabales

During this survey on the ubiquity of the phytochelatin metal inactivating pathway a cell culture of *Glycine max* (soybean) was tested. This member of the order Fabales (Leguminosae) also yielded inducible, metal containing, low molecular weight peptides. This material was subjected to HPLC analysis and displayed a partition pattern similar to that of phytochelatins. However, the sulfhydryl-rich peptides had a slightly increased retention time compared to the usual phytochelatins (45 sec for PC_2) [4]. Automated amino acid analysis of the hydrolyzed peptide revealed that these peptides are composed only of glutamic acid, cysteine and a non-protein amino acid which was identified by mass spectroscopy to be β-alanine [4]. These peptides were unequivocally identified as homologues of the phytochelatins by chemical analysis. The chain length pat-

Table IV. Phytochelatin synthesis in members of the Monocotyledoneae; list abbreviated from a survey of 23 species.

Species	Order	*	+	PC_2[a] [nmol/mg	PC_3 dwt]	PC_4	PC_5	Total PC[b]
Lemna gibba	Arales	D	H	0.54	0.67	0.20	0.00	3.89
Phoenix dactylifera	Arecales	D	H	1.10	0.61	0.28	0.01	5.18
Asparagus officinales	Asparagales	D	H	1.82	0.33	0.09	0.00	4.97
Ananas comosus	Bromeliales	S	DAX	0.86	0.24	0.07	0.00	2.71
Commelina graminifolia	Commelinales	D	H	0.31	0.32	0.08	0.00	1.89
Cyperus esculentus	Cyperales	D	H	0.84	0.68	0.27	0.11	5.53
Triticum aestivum	Poales	D	H	0.93	0.74	0.07	0.00	4.38
Musa ensete	Zingiberales	D	H	0.47	0.30	0.09	0.05	2.42

[a, b, *, +]: as in Table II.

tern of these compounds is identical to the PC series. The only difference resides in the carboxy terminus where β-alanine substitutes for glycine. These compounds were called "homo-phytochelatins" (h-PC) and display the general formula (γ-Glu−Cys)−β-Ala. Members for $n = 2-7$ have been found up to now. A limited survey [4] proved that in the order Fabales there are species producing upon exposure to heavy metal exclusively h-PC, others only PC and some both h-PC and PC within one and the same plant. The carboxy terminal amino acid is obviously determined by the presence of either glutathione, or homo-glutathione or both within a given plant species, the tripeptides being assumed to be the biosynthetic precursors of the PC families [3, 4, 15]. The survey of differentiated plants or cell cultures as to their ability to form GSH or h-GSH, as well as to synthesize upon Cd^{2+} exposure PC (only PC_2 shown) or h-PC (only h-PC_2 shown) has now been extended and is shown in Table V. The total content of (γ-Glu−Cys) units is given as a measure of the PC synthesizing capacity of the individual plant species. A total of 57 species of 36 genera of all the eleven tribes of the Fabaceae, 6 species of 4 tribes of the Caesalpiniaceae, and 4 species of 4 tribes of the Mimosaceae have been investigated. While all investigated species of the Mimosaceae and Caesalpiniaceae, as well as all analyzed species of the tribes Genisteae, Podalyrieae and Sophoreae synthesized PC's, the analyzed members of the tribus Phaseoleae produced h-PC's as well. Only *Clitoria ternatea* and some *Vigna* species within the Phaseoleae synthesize GSH/PC. Both, *Vigna umbellata* and *Vigna radiata* (syn. *Phaseolus aureus*) synthesize h-

Table V. Synthesis of phytochelatin (PC) and homo-phytochelatin (h-PC) in members of the order Fabales containing GSH and/or h-GSH.

Species	Tribus	GSH [nmol/mg dwt]	h-GSH	PC_2[a]	h-PC_2	Total PC[b]
Caesalpiniaceae						
Tamarindus indica	Amherstieae	0.36	0	0.03	0	0.06
Bauhinia purpurea	Bauhinieae	1.06	0	0.17	0	0.48
Caesalpinia sappan	Caesalpinieae	1.35	0	0.57	0	2.58
Cassia angustifolia	Cassieae	2.55	0	0.91	0	4.66
Mimosaceae						
Acacia karroo	Acacieae	4.11	0	0.11	0	0.21
Neptunia oleracea	Adenanthereae	0.82	0	0.14	0	0.35
Albizzia lophanta	Ingeae	1.70	0	0.14	0	0.68
Mimosa pudica	Mimoseae	0.67	0	0.17	0	1.03
Fabaceae						
Onobrychis viciifolia	Coronilleae	1.69	0	0.61	0	4.14
Lonchocarpus violaceus	Dalbergieae	2.61	0	0.23	0	0.71
Pterocarpus officinalis	Dalbergieae	0.31	1.10	0.04	0.06	0.25
Astragalus gummifer	Galegeae	1.06	0.20	0.20	0.15	2.67
Astragalus preussii	Galegeae	2.84	0	0.72	0	2.02
Crotalaria crassipes	Genisteae	1.71	0	0.57	0	4.68
Lotus ornithopodioides	Loteae	0.85	0.85	0.13	0.04	0.70
Ononis natrix	Ononideae	0.60	0.31	0.07	0.07	0.48
Canavalia ensiformis	Phaseoleae	0.68	0.68	0.21	0.21	1.62
Clitoria ternatea	Phaseoleae	1.01	0	0.49	0	1.46
Erythrina coralloides	Phaseoleae	0	1.78	0	1.24	4.37
Baptisia australis	Podalyrieae	1.21	0	0.07	0	0.80
Sophora japonica	Sophoreae	1.48	0	0.71	0	2.66
Melilotus alba	Trifolieae	1.20	1.20	1.84	1.84	20.33
Lathyrus ochrus	Vicieae	2.38	0.71	0.56	0.50	3.50

[a]: Indices as in Table I.
[b]: Total amount of (γ-Glu−Cys) residues induced. Numbers are higher than the sum of PC_2 + h-PC_2 due to synthesis of higher homologue which are not listed here. Neither phytochelatin nor homo-phytochelatin peptides were detected in control plants not exposed to Cd^{2+}.

GSH and therefore h-PC. In all other tribes, *i.e.* Galegae, Vicieae, Trifolieae, Loteae, Ononideae, and Dalbergieae (with very few exceptions) only the simultaneous presence of GSH/ h-GSH and therefore PC/h-PC was observed. In the tribe Coronilleae only one of five species analyzed had the mixed PC/h-PC type, all the others contained only GSH/PC. The consistency of the monomer/oligomer pattern is strong evidence for the direct participation of GSH and h-GSH in the biosynthesis of PC and h-PC, respectively.

Discussion

In this survey somewhat more than 200 individual species of lower and higher plants were tested for their ability to form phytochelatin or homo-phytochelatin heavy-metal binding complexes after exposure to cadmium ions. Indeed, this survey represents less than 0.1% of all existing plants. But one or several members of all the major plant classes, orders and families were investigated and not a single plant species was found which did not respond with either PC or h-PC synthesis to exposure to the heavy metal. The (γ-Glu−Cys) oligomer containing a glycine carboxy terminus is by far the predominant heavy-metal binding complex. The members with a β-alanine terminus are exclusively restricted to the order Fabales of the Angiospermae. This survey made it clear that there is no other major complex involving (γ-Glu−Cys) oligomers with an amino acid carboxy terminus other than glycine or β-alanine. It made also clear that the glycine-deficient phytochelatin species $(Glu-Cys)_2$ first found in maize [20], which also occur in the fungi *Schizosaccharomyces pombe* and *Candida glabrata* [6], are not of general occurrence. This des-glycine phytochelatin may very well be a degradation product of normal phytochelatins by action of carboxy peptidases.

Animals respond to heavy metal stress by induction of metallothionein through gene expression. Plants react by induction of phytochelatin (homo-phytochelatin) formation *via* enzymic polymerization of glutathione (or homo-GSH). Thus an evolutionary divergence in heavy metal sequestration has occurred between plants and animals. In this context, it is of considerable interest that for the first time a fungus *(Candida glabrata)* was found [6] which expresses both metallothionein and phytochelatin. The biosynthesis of metallothioneines is regulated by Cu^{2+} while phytochelatin is induced by Cd^{2+}. This finding [6] that the fungus *Candida glabrata* contains metal inactivating mechanisms of both the plant and animal type may reflect lateral gene transfer in the division Mycophyta (fungi). This division should in the future be further analyzed for the metal inactivating mechanisms.

Winge and his coworkers [21] recently critically discussed the naming of the $(\gamma\text{-Glu}-Cys)_n-Gly$ or $(\gamma\text{-Glu}-Cys)_n-\beta\text{-Ala}$ peptides. They [6, 21] prefer to call these compounds γ-glutamyl peptides, a name which is certainly not tenable since γ-glutamyl compounds occur abundantly in the plant kingdom [23] and the generic name can thus not be used to classify these specific types of metal-binding $(\gamma\text{-Glu}-Cys)_n-Gly$ or $-\beta\text{-Ala}$ oligomers. Previously, these peptides have been referred to as "cadystin A and B" [5]. Independently, after the discovery of the families of both types of oligomeric peptides as principal heavy-metal chelating substances in plants, we proposed the name "phytochelatins" [3]. The subsequent criticism was that these names are "either non-descriptive or too restrictive" [21]. The restrictiveness was meant to refer to the occurrence of these compounds solely in plants. Winge *et al.* [24] distinguish three separate kingdoms (plants, fungi and protista) in which phytochelatins are found and argue that therefore the name *phyto*-chelatins is not appropriate. These authors obviously refer to suggestions by Whittaker [25] who postulated a total of five (though overlapping) organismic kingdoms, ideas which are controversially discussed by taxonomists since. Photosynthesizing algae (even those which do it facultatively like *Euglena* [7]) are part of the plant kingdom and the overwhelming majority of taxonomists regard fungi as a specific organisational type of the plant kingdom (*e.g.* [26]). There is little doubt that some fungi are evolutionarily more closely related to plants while others are more closely related to animals [27]. It might be suggested here that the mode of metal chelation may even provide a clue to the evolutionary origin of a given fungal species. We have to assume now that over 300,000 organisms of the plant kingdom possess the principle of metal homeostasis and detoxification through the formation of the chelating compounds $(\gamma\text{-Glu}-Cys)_n-Gly$ or $-\beta\text{-Ala}$; we therefore think that the name phytochelatins (homo-phytochelatins) for these types of compounds is amply justified.

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