REVIEW

# The role of phytochelatins in plant and animals: A review

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**The role of phytochelatins in plant and animals: A review**

Phytochelatins (PCs) are thiol-containing oligomers formed in post-translational synthesis from glutathione. They were firstly described in yeasts Schizosaccharomyces pombe. Subsequently their presence was monitored in plants, microorganisms, but also in many animal species. It is well known, that in plants PCs exhibit significant function in manner of chelating of metals. Since they contain thiol functional groups originated from cysteine moieties they keep a metal homeostasis balanced. Although the presence of genes encoding PCs was confirmed in a few animal species, their function in these organisms was not satisfactorily elucidated. Some studies revealed that PCs in animal species are closely linked with detoxification processes in similar way as in plants. It was also shown that thiols in invertebrates are utilized as the biomarkers of heavy metals contamination.

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## 1. Phytochelatins and phytochelatin synthase

Increasing emissions of heavy metals such as cadmium, mercury, and arsenic into the environment pose an acute problem for all organisms. As a mass of protection, many of them, develop mechanisms of full resistance or at least exhibit partially resisting toward these effects. In this way, based on the chemical similarity of the involved metallic species, they are able, to replace them with viable metals necessary for the effective functioning of the cell. These heavy metals may be bound to the functional groups of proteins and modify their structure and through this also affect their physiological function[1,2]. Higher plants, algae, certain yeasts and animals are able to respond to heavy metals by synthesizing phytochelatins (PCs) and related cysteine-rich polypeptides. Phytochelatin synthases are γ-glutamylcysteine (γ-Glu-Cys) dipeptidyl transpeptidases that catalyze the synthesis of heavy metal-binding PCs[3,4]. PCs, cysteine-rich peptides, are produced from glutamine, cysteine and glycine. Unlike commonmetal-binding structures, MT and GSH, PCs are not gene-encoded, but enzymatically synthesized peptides[5]. PCs have been identified in a wide variety of plant species, microorganisms and some invertebrates[6-10]. They are structurally related to glutathione (GSH) and were presumed to be the products of a biosynthetic pathway. Numerous physiological, biochemical and genetic studies have confirmed GSH as the substrate for PCs biosynthesis[11,12]. The general structure of PCs is (c-Glu-Cys)*n*-Gly, with increasing repetitions of the dipeptide Glu-Cys linked through a c-carboxylamide bond (**Fig 1**), where n varies from 2 to 11, but typically reaching not further than five[13]. Except glycine, also other amino acid residues can be found on C-terminal end of $(\gamma\text{-Glu-Cys})_n$ peptides. Examples of which, like Ser, Glu, Gln and Ala are often found at

this position in some plant species, and they are assumed to be functionally analogous and synthesised via essentially similar biochemical pathways[14, 15]. *In in vitro* studies of PC synthase expressed in *E. coli* or in *S. cerevisiae*, the enzyme was activated to varying extents by Cd, Cu, Ag, Hg, Zn and Pb ions[16-18]. PC synthase genes were also isolated in *A.thaliana*[16] and *T.aestivum*[18]. Genes homologous to those from A.thaliana and *T.aestivum* were also found in *S.pombe* and *C.elegans*, suggesting the existence of PC synthase genes in more species[19].

## 3. Phytochelatins in plants

Contamination of soils with toxic metals has often resulted from human activities, especially those related to mining, industrial and emissions. In this context, phytoremediation has been developed as a cost effective and environmentally friendly remediation method of contaminated soils[22, 23]. In recent years many studies showed the mechanisms of chelation of metals-PC[24-28]. Chelation and sequestration of metals by particular ligands are also mechanisms used by plants to cope with metal stress.

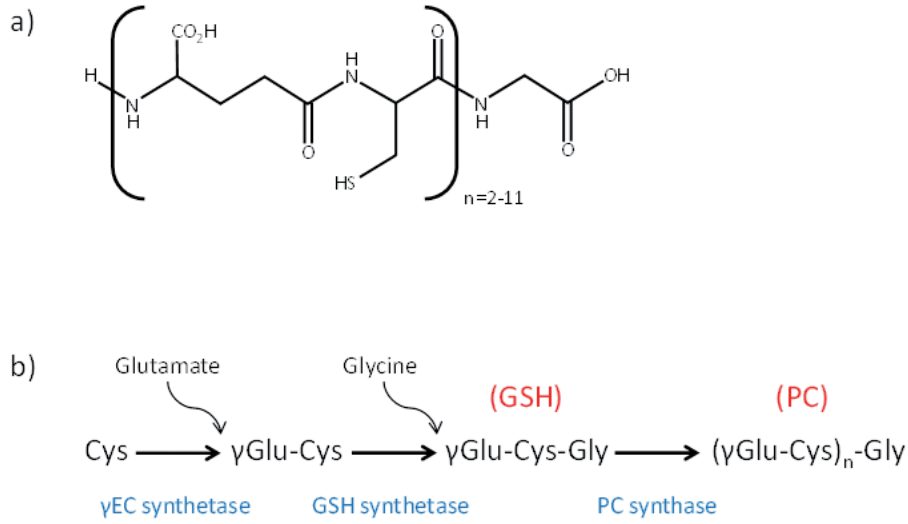


**Figure 1.** a) The general structure of PCs. b) Biosynthesis of phytochelatins.

## 2. Phytochelatins inmicroorganisms

Interestingly, although PC(*n*=2) has been described in the yeast *S. cerevisiae*, there is no homologue of the PC-synthase genes in the *S. cerevisiae* genome. An alternative pathway for PCs biosynthesis which has been in *S. pombe* has been proposed. However, it can be a similar pathway that functions in *S. cerevisiae* too[20]. A study shows that the two vacuolar serine carboxypeptidases are responsible for PC synthesis in *S. cerevisiae*. Therefore, the finding of a PCS-like activity of these enzymes in vivo discloses another route for PC biosynthesis in eukaryotes[21].

The two best-characterized metal-binding ligands in plant cells are the PCs and metallothioneins [29-31]. Naturally hyperaccumulating plants do not overproduce PCs as part of their mechanism against toxic metals and this feature appears to be an inducible rather than a constitutive mechanism, observed especially in metal non-tolerant plants[8].

Several studies of plants that overexpressed γ-glutamyl-cysteine synthetase or transgenic plants expressing bacterial γ-glutamyl-cysteine synthetase evaluated its effect on metal tolerance based on the assumption that higher levels of GSH and PCs will lead to more efficient metal sequestration[32]. *Bacopa monnieri,* a wetland

macrophyte is well known for its accumulation potential of metals and metal tolerance and thus is suitable in phytoremediation. Aquatic plants respond to metal stress by increased production of PCs as well as other antioxidants. *B. monnieri* is wellknown for the accumulation potential of various heavy metals and warrants its evaluation for metal tolerance and detoxification mechanism for its suitability in phytoremediation[9]. *Arabidopsis thaliana* showed that Cd is immediately scavenged by thiols in root cells, in particular PCs, at the expense of GSH. At the same time, a redox signal is suggested to be generated by a decreased GSH pool in combination with an altered GSH:GSSG ratio in order to increase the antioxidant capacity[24]. Overexpression of PCs synthase in Arabidopsis led to 20-100 times more biomass on 250 and 300 μM arsenate than in the wild type. Also, the accumulation of thiol-peptides was 10 times higher when after the exposure to Cd and arsenic, compared to the wild type. Gamma-glutamyl cysteine, which is a substrate for PC synthesis, increased rapidly, after arsenate or cadmium exposure. Overexpression of this gene can be useful for phytoremediating[33]. Also, Legumes are also capable for synthesising homophytochelatins in response to heavy metal stress[32]. Citrus plants were able to synthesize PCs in response to heavy metal intoxication[26]. In wheat, PCs–heavy metal complexes have been reported to accumulate in the vacuole. Retention of Cd in the root cell vacuoles might influence the symplastic radial Cd transport to the xylem and further transport to the shoot, resulting in genotypic differences in grain Cd accumulation[34].

## 4. Phytochelatins in animals

PCs proteins have been broadly described and characterized in plants, yeasts, algae, fungi and bacteria, as well as nematodes and trematodes[35]. PC synthase genes are also present in animal species from several different phyla. PCs synthesis appears not to be transcriptionally regulated in animals[36]. Originally it is thought to be found only in plants and yeast, but PC synthase genes have been found in species that span almost the whole animal tree of life.

### 4.1 Fuctionsl of PCs in animals

Biochemical studies have also shown that these PCS genes are functional: the *Caenorhabditis elegans* PC synthase produces PCs when it is expressed in an appropriate host, and knocking out the gene increases the sensitivity of *C. elegans* to cadmium[37]. In several studies PCs have been measured by direct biochemical analysis of *C. elegans* tissue extracts, and found that cadmium exposure did indeed increase PCs levels in *C. elegans*. $PC_2$, $PC_3$, and $PC_4$ have all been found, with PC2 the highest concentration[6, 38, 39]. Therefore, these studies showed conclude that PCs production plays a major role in protecting *C. elegans* against cadmium toxicity. $PC_2$ and $PC_3$ were increased in autochthonous *Lumbricus rubellus* populations sampled from contaminated sites[36].

The yeast (i.e. *S. pombe*) possesses an ATP-binding cassette (ABC) transporter, Hmt1, which was originally thought to play a possible role in translocation of PCs–metal complexes to the vacuole. However, while knocking out the *C. elegans* HMT-1 (CeHMT-1) the sensitivity toward cadmium does increase, and the increase is greater than could be explained by a lack of PCS alone[40].

It is important to say that MTs are widely established as a key metal detoxification system in animals, even though they certainly have many other biological functions as well. Until now, there is very little known about how MTs and PCs may complement each other for dealing with toxic metals[36].

## 5. Methods for phytochelatins determination

Recently, *Wood et al.*, showed the analytical methodology for quantification of PCs and their metal(loid) complexes[41]. The classical approach to the analysis of PCs is by reversed phase HPLC with post-column derivatization of the sulfhydryl groups and spectrophotometric detection (but this is not specific to PCs). Independent studies showed a sensitive method for determination of PCs by HPLC with fluorescence detection[42, 43]. A simple sensitive method for the identification, sequencing and quantitative determination of PCs in plants by electrospray

tandem mass spectrometry (ESI MS-MS) was showed in different studies[44, 45]. Other study showed the combination of three process for identification PCs: (1) simple sample preparation including thiol reduction, (2) rapid and high resolution separation using ultra-performance liquid chromatography (UPLC), and (3) specific and sensitive ESI-MS/MS detection using multi-reaction mode (MRM) transitions in alga's extract[46]. Zitka et al., optimized high performance liquid chromatography coupled with electrochemical detector for determination of PC2[47].

## 6. Methods for phytochelatin synthase determination

High performance liquid chromatography coupled with electrochemical detector was to suggest as a new tool for determination of the PCs synthesis activity[48]. The optimized procedure was subsequently used for studying PC synthase activity in the tobacco BY-2 cells treated with different concentrations of Cd (II) ions and the results were in good agreement with Nakazawa et al.,[49]. Another study in animals showed that HPLC-LC system coupled to a single quadrupole LC–mass spectrometer equipped with electrospray ionization was sensitive method for determination of PCs synthesis activity [35]. A high sensitive assay for PCS activity was devised, in which the dequenching of Cu(I)-bathocuproinedisulfonate complexes was used in the detection system of a reversed-phase high-performance liquid chromatography. The present assay method is a sensitive tool that can be used to investigate this issue and would allow determination of PCS activity using 10–100-fold less protein[50].

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