Chapter

Phytochelatin Synthase in Heavy Metal Detoxification and Xenobiotic Metabolism

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Abstract

Phytochelatin synthase (PCS) is well-known for its role in heavy metal detoxification in plants, yeasts and non-vertebrate animals. It is a protease-like enzyme that catalyzes glutathione (GSH) to form phytochelatins (PCs), a group of Cys-rich and non-translational polypeptides with a high affinity to heavy metals. In addition, PCS also functions in xenobiotic metabolism by processing GS-conjugates in the cytosol. Because PCS is involved in GSH metabolism and the degradation of GS-conjugates, it is one of the important components in GSH homeostasis and GSH-mediated biodegradation. This chapter reviews the biochemical mechanism of PCS, how the enzyme activity is regulated, and its roles in heavy metal detoxification as well as GS-*S*-conjugate metabolism. This chapter also highlights the potential applications of PCS in the improvement of plant performance under combined stresses.

Keywords: Phytochelatin synthase, heavy metal stress, GS-conjugate metabolism, glutathione, combined pollution

1. Introduction

Phytochelatins (PCs, $(\gamma Glu\text{-}Cys)_n$-Gly, n = 2–11) are cysteine-rich polypeptides that are synthesized non-translationally from the tripeptide glutathione (GSH, γGlu-Cys-Gly); this process is catalyzed by phytochelatin synthase (PCS, EC 2.3.2.15) [1–4]. PCs play essential roles in heavy metal detoxification because of their high affinities to a broad range of metal ions, e.g. cadmium (Cd), mercury (Hg), arsenic (As), zinc (Zn), lead (Pb), silver (Ag), nickel (Ni) and copper (Cu) [1–3]. Upon exposure to heavy metals, PCs are synthesized in the cytosol to chelate free metal ion and to prevent the generation of hydroxyl radicals [4–6] (**Figure 1**). These PC-metal complexes eventually are transferred into the vacuole through specific tonoplast ABCC-type transporters for sequestration [22–26] (**Figure 1**). In plants, PCS is constitutively expressed in the cytosol and can be activated by multiple types of metal ions [1, 3, 6]. For example, AtPCS1 isolated from *Arabidopsis thaliana* can be activated by the metal ions mentioned above [6]. In addition, some PCS homologs, such as the model legume *Lotus japonicus* LjPCS1 and LjPCS3, can be activated by iron (Fe) and aluminum (Al) [27].

PCS can be found in plants, yeasts and non-vertebrate animals and plays a critical role in responding to heavy metal stress in these organisms [28–32]. It was first partially purified from the suspension cells of bladder campion (*Silene cucubalus*) for its ability to synthesize PCs from GSH in the presence of Cd^{2+} [4]. Soon after the isolation of the enzyme, the genes coding PCS were cloned from plant and yeast

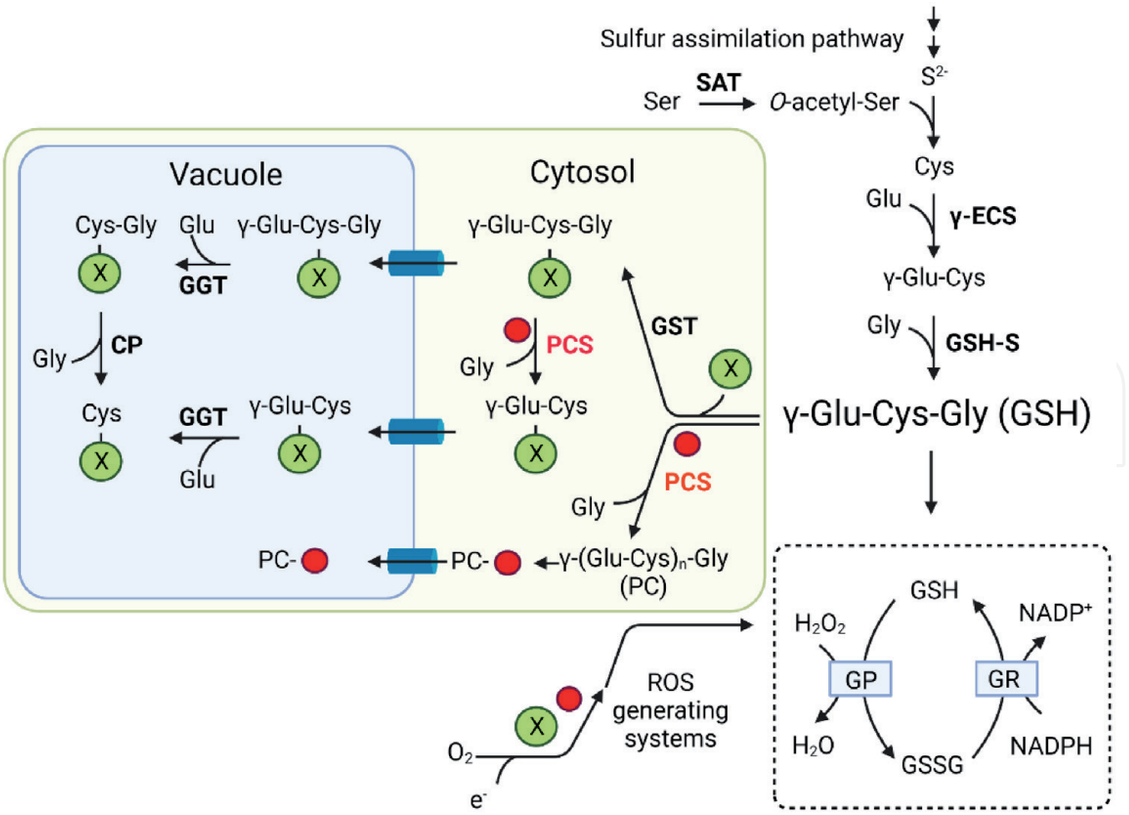

Figure 1.
The involvement of phytochelatin synthase in glutathione metabolism, heavy metal detoxification, and glutathione-S-conjugate degradation.
An overview of the roles of phytochelatin synthase (PCS) in the metabolic pathways of glutathione (GSH, γ-Glu-Cys-Gly). The brief pathway of GSH biosynthesis and the major route of glutathione-S-conjugate (GS-conjugate) degradation are also shown in the figure [7–9]. The presence of xenobiotic compounds (X, marked as green circles) and free heavy metal ions (red circles) induces ROS generation and causes oxidative stress. The cytosolic xenobiotic compound is transferred to GSH by glutathione S-transferase (GST) to initiate the detoxification, and then the GS-conjugates enter vacuoles for further degradation [10–13]. In the vacuoles, GS-conjugates are first catalyzed to Cys-Gly-conjugates by γ-glutamyl transpeptidase (GGT) before the final deglycination catalyzed by carboxypeptidase (CP) [14–16]. In the presence of heavy metals, PCS uses GSH as substrates to synthesize phytochelatins (PCs), which chelate free metal ions in the cytosol [4]. Heavy metals also activate PCS to initiate the cleavage of GS-conjugates [17–20]. The cytosolic γ-Glu-Cys-conjugates then enter vacuoles and serve as the substrates of GGT for the second step of degradation [21]. The blue cylinders represent tonoplast ABCC transporters that facilitate the import of GS-metabolites and PC-metal complexes [12, 13, 22–24]. In the brief biosynthetic pathway of GSH, the rate-limiting enzymes are indicated in bold. Note that the cellular compartmentation of GSH synthesis or redox reactions is not included in this figure. SAT, serine acetyltransferase; γ-ECS, γ-glutamylcysteine synthetase; GSH-S, GSH synthetase. GP, GSH peroxidase; GR, GSH reductase. The figure was created with BioRender.com.

sources, including AtPCS1, TaPCS1 from wheat (*Triticum aestivum*), and SpPCS from *Schizosaccharomyces pombe* by three independent research groups [5, 33, 34]. Since then, PCS sequences from various model organisms have been largely characterized, such as *Caenorhabditis elegans* (CePCS1) [31], the Cd hyperaccumulator *Thlaspi caerulescens* (TcPCS1) [35], and *Oryza sativa* (OsPCS1, OsPCS2, OsPCS5, OsPCS15) [36–38]. Besides eukaryote PCS sequences, a gene encoding a PCS-like protein, NsPCS, was identified from the genome of cyanobacterium *Nostoc* sp. [39–42].

PCS is a key component for the heavy metal tolerance in plants. Its importance was first confirmed in the Arabidopsis mutants locking AtPCS1 activity, as these mutants show severe growth defects when challenged by heavy metals such as Cd^{2+}, Hg^{2+}, Zn^{2+}, Pb^{2+}, and As^{3+} [28, 43–45]. The synthesis of PCs is crucial to the local response to heavy metal stress and is also involved in the roots-to-shoots translocation of heavy metals [26, 37, 43, 46]. The first evidence of the long-distance transfer of PC-metal complexes is that the PCs synthesized in the roots can be translocated to the shoots via phloem loading and *vice versa* [26, 43, 46]. Additionally, plants defective in PC

synthesis show altered patterns of heavy metal accumulation at the whole-plant level while being sensitive to heavy metal stress. For example, the Arabidopsis AtPCS1-deficient mutant, *cad1–3*, accumulated significantly less Cd in the shoots than the wild type or the transgenic line heterologously overexpressing TaPCS1 [43], and the rice *OsPCS2* RNAi plants failed to transfer As^{3+} from the roots to the shoots [37]. Overall, the phenotypes of these PCS-deficient mutants suggest heavy metal ions absorbed through the roots can be loaded into the shoots in the form of PC-chelates.

PCS is a well-known multitasker involved in different biological processes [21, 47]. Besides its significant role in synthesizing PCs from GSH, PCS can catalyze the deglycination of GSH-*S*-conjugates (GS-conjugates), and thus, it is involved in the GS-conjugate catabolism [17–21, 48]. In addition, PCS is also associated with indole glucosinolates metabolism and immune responses [49–51]. Intriguingly, the catalytic-site mutants of PCS are still functional in this pathway, which suggests that the role of PCS in the indole glucosinolate metabolism is independent of PC synthesis and GS-metabolism [51]. Among these PCS-involving biological processes, this chapter focuses on the catalytic mechanism of PCS and its functions in both heavy metal stress and GSH metabolism. The potential applications of PCS in combating multiple stresses are also discussed.

2. The biochemical mechanism of phytochelatin synthase

2.1 The domain organization of phytochelatin synthase

The eukaryotic PCS has two domains with distinct functions: a conserved N-terminal domain that shows γ-glutamylcysteine dipeptidyl transpeptidase activity and a variable C-terminal domain involved in metal sensing [52–54]. Using AtPCS1 as a model, the molecular functions of the N- and C-domains as well as the catalytic mechanism of eukaryotic PCS were revealed [6, 53–55]. The N-terminal half AtPCS1 is sufficient for deglycination of GSH and elongating PC molecules, indicating that the N-terminal domain carries out the core catalysis [53, 56]. However, the truncated AtPCS1 without the C-terminal domain is less thermostable and has lower PC synthetic activity than the full-length enzyme [53, 55, 56]. Notably, the deletion of the C-terminal domain completely impairs the PC synthesis activity of the enzyme in the presence of Zn^{2+} and partially inactivates PC synthesis in the Cd- or Hg-containing reactions [53, 55]. These findings suggest that the C-terminal domain is essential for stabilizing the protein and functions as a metal sensor [53, 55]. More evidence has shown that the C-terminal end of AtPCS1 is required for the augmentation of PC synthetic activity. One example is that the residues from Asp373 to the C-terminal end of AtPCS1 contain multiple regions involved in Zn-dependent and As-dependent activation of PC synthesis [45, 57, 58]. (Also see Section 2.4: The activation of phytochelatin synthase through the chelation of heavy metal ions).

PCS-like sequences also exist in prokaryotes with moderate sequence homology to the N-domain of eukaryotic PCS [39–41]. However, prokaryotic PCS likely has unique functions apart from PC synthesis. For example, the PCS homolog found in cyanobacterium *Nostoc* sp. PCC 7120 (NsPCS) shows distinct characters from its eukaryotic counterparts that efficiently catalyze PC synthesis. NsPCS is a "half-PCS molecule" that does not have a C-terminal domain [39, 40, 52] and catalyzes the hydrolysis of GSH at a high rate and the synthesis of PCs at a relatively low rate [39, 40, 42]. Besides, the enzyme activity of NsPCS seems insensitive to the absence or presence of Cd^{2+}, which suggest that the prokaryotic PCS is involved in GSH metabolism in the cells rather than the responses to heavy metal stress [39, 42].

2.2 The core catalytic mechanism

Vatamaniuk et al. [6] first confirmed that the synthesis of PCs occurs through a ping-pong mechanism and involves two substrates: one GSH as the low-affinity substrate for the first step of PC synthesis and one metal-GSH conjugate as the high-affinity substrate for the second step [6]. In the standard PC synthesis reactions *in vitro*, which resemble the concentrations of the GSH and metal ions in the cytosol, GSH exists at a considerably higher level (millimolar) than heavy metal ions (micromolar) [6, 7, 59]. Presumably, more than 98% of total metal ions in this condition are associated with GSH as bis(glutathionato)metal ions (metal·GS_2), and the free Cd concentration can be as low as 10^{-6} μM [6]. Under these circumstances, GSH and Cd·GS_2 are two separate compounds for PC synthesis.

Right after GSH enters the catalytic site of PCS, a Gly residue is removed to form the γGlu-Cys acyl-enzyme intermediate, and then a metal-GS_2 accepts the γGlu-Cys unit to generate a PC_2 ((γGlu-Cys)$_2$-Gly) [6, 21, 54, 55]. Following the synthesis of PC_2, the elongation of PCs occurs using previously synthesized PCs as acceptors to receive γGlu-Cys [6, 21, 54, 55, 60]. The whole process can be described as two equations:

$$PCS + \gamma\text{Glu} - Cys - Gly \rightarrow PCS - \gamma\text{Glu} - Cys + Gly \tag{1}$$

$$\begin{aligned} PCS - \gamma\text{Glu} - Cys + \text{metal}\cdot\left(\gamma\text{Glu} - Cys - Gly\right)_2 &\rightarrow PCS \\ + \text{metal}\cdot\left(\gamma\text{Glu} - Cys\right)_2 - Gly + \gamma\text{Glu} - Cys - Gly & \end{aligned} \tag{2}$$

Overall, PCS catalyzes the peptide chain elongation from C-to-N terminus [4, 6, 21, 54]:

$$\begin{aligned} \left(\gamma\text{Glu} - Cys\right) - Gly + \left(\gamma\text{Glu} - Cys\right)_n - Gly \\ \rightarrow \left(\gamma\text{Glu} - Cys\right)\cdot\left(\gamma\text{Glu} - Cys\right)_n - Gly + Gly \end{aligned} \tag{3}$$

PCS and Cys proteases share similar core catalytic mechanisms to hydrolyze a GSH molecule and form a γ-Glu-Cys-acyl–enzyme intermediate [41, 52]. The Cys protease-like catalytic triad of PCS was confirmed based on the mutagenic studies of AtPCS1 and the crystal structures of NsPCS [41, 54, 55]. Vatamaniuk et al. and Romanyuk et al. reported that Cys56, His162, and Asp180 of AtPCS1 are the three residues of the catalytic triad among divergent PCS sequences [54, 55]. The molecular interaction between these residues and GSH was further revealed by Vivares et al. with the crystal structures of native NsPCS and the γ-Glu-Cys-acyl–enzyme intermediate at a 2.0-Å resolution [41]. Although NsPCS only shares 36% identity at the amino acid level with AtPCS1, it contains the conserved catalytic triad and can catalyze the deglycination of GSH [39–42]. These crystal structures provide details about the hydrolysis of the peptide bond that involves Cys56 and the 3D structure of the Cys-His-Asp catalytic triad [41]. It is worth mentioning that NsPCS formed homodimers in the crystallization experiments [26]. This is in agreement with the dimerization of the partially purified PCS from *Silene cucubalus*, which was confirmed by determining the native molecular weight of the protein [4].

2.3 Critical amino acids contributing to the enzyme activity

Based on the high-resolution crystal structure of NsPCS, multiple research groups have simulated putative 3D structures of eukaryotic PCS using various programs, and these structure models provide valuable information that uncovers

the conserved molecular mechanism of PCS [56, 61–65]. For example, the molecular models of AtPCS1 reveal the key amino acids that contribute to the mechanism of the second substrate recognition and the enzyme activation through Thr phosphorylation [56, 61]. Chia et al. first reported how AtPCS1 might attract and stabilize the second substrate, metal-GS_2, after the γ-Glu-Cys-acyl–enzyme intermediate is formed [61]. In this study, the modeled AtPCS1 structure revealed a pocket in proximity to the first substrate-binding site, consisting of three loops containing several conserved amino acids, including Arg152, Lys185, and Tyr55. Mutations on Arg152 or Lys185 (Arg-to-Lys or Lys-to-Arg substitutions) resulted in the complete abrogation of enzyme activity, indicating that the arrangement of these positive charges is crucial for the binding of the second substrate. Mutations at Tyr55 did not completely impair the enzyme activity, but the Tyr55 mutant AtPCS1 showed lower catalytic activities than the wild-type enzyme due to a reduced affinity to metal-GS_2. In addition, the mutation at Tyr55 reduced Cd^{2+} binding ability of the AtPCS1 protein. It was therefore suggested that Tyr55 binds to the Cd ion of metal-GS_2 through cation-π interaction and thus contributes to the recognition of the second substrate. Besides these three amino acids, other conserved residues on the loops constituting the second substrate-binding pocket, including Gln50, Glu52, Glen157, Phe184, and Tyr186, are also important for the PC synthesis activity [61, 62].

Wang et al. identified that Thr49 is the phosphorylation site related to the activation of AtPCS1 [56]. The mutant AtPCS1 with Thr49-to-Ala49 substitution could not be phosphorylated, and its PC synthesis activity was significantly lower than that of the wild-type enzyme. According to the proposed 3D model of AtPCS1, Thr49 is within proximity to Arg183, which is also crucial for the catalytic activity of AtPCS1, and both residues are next to the catalytic site and substrate binding pockets. It was proposed that the phosphorylated Thr49 interacts with Arg183, and that this interaction serves as a "molecular clip" to give the active site a conformation appropriate for catalysis. Because Thr49 and Arg183 are both highly conserved among PCS sequences, the activity of eukaryotic PCS may as well be regulated by similar phosphorylation modifications [56, 66].

2.4 The activation of phytochelatin synthase through the chelation of heavy metal ions

As a key component of early response to heavy metal stress, PCS protein is constitutively expressed in the cytosol for rapid activation stimulated by heavy metals [2, 3, 6]. The heavy metal ions entering cytosol are essential for forming the second substrate [6, 60]. They can also bind to PCS, resulting in augmentative activation [6, 55, 67, 68]. Moreover, heavy metals could be a critical factor that triggers PCS phosphorylation [56, 69]. For example, AtPCS1 phosphorylation only occurred in the presence of Cd^{2+} in the *in vitro* experiments [56].

PCS, confirmed to be a metalloenzyme *in vitro*, is also likely to be one *in vivo* [6, 17]. Equilibrium analyses show that one AtPCS1 molecule binds seven Cd^{2+} in solutions containing 10 μM $CdCl_2$ [6, 61]. Apart from Tyr55, which is proposed to bind the Cd^{2+} on the metal-GS_2, the Cd binding capability of PCS presumably comes from conserved Cys pairs and CysXXCys motifs also found in metallothionein [30, 61]. Peptide screening of SpPCS and TaPCS showed that the core sequences containing consensus Cys-rich motifs could bind Cd^{2+} *in vitro* [67]. The subsequent site-direct mutagenesis analysis indicated that conserved Cys pairs at the N-terminal domain were critical for PCS activity, while the Cys-rich motifs at the C-terminal domain only slightly affected the PC synthesis rate [67]. It is not yet clear how these

Cys-rich motifs enhance the PC synthesis rate. It is possible that they bind metal-GS_2 complexes or free metal ions to stabilize the protein structure [6, 30, 55, 67]. More investigations are still needed to explain the molecular functions of these Cys-rich motifs and how they participate in the metal activation of PCS.

3. Phytochelatin synthase-targeting genetic engineering approaches in phytoremediation of heavy metals

3.1 The effects of phytochelatin synthase overexpression on the accumulation of heavy metals in plants

PC synthesis plays a critical role in heavy metal tolerance and accumulation. It is therefore no surprise that the breeding and engineering approaches for phytoremediation requiring heavy metal hyperaccumulators have focused on strategies to enhance PC biosynthetic capacity [48, 70–76]. Studies have shown that the transgenic plants expressing functional PCS usually have a higher tolerance to heavy metal stress. For example, the overexpression of AtPCS1 in Arabidopsis, tobacco or Indian mustard (*Brassica juncea*) enhanced Cd, Zn, and As tolerance and accumulation [71, 77–80]. Other PCS homologs e.g., CePCS, TaPCS1, NtPCS1, CdPCS from an aquatic As-accumulator plant (*Ceratophyllum demersum*), MaPCS1/MaPCS2 from mulberry (*Morus alba*), and VsPCS1 from legume *Vicia sativa* were also used to develop transgenic plants that accumulate higher concentrations of heavy metals than their natural variants [43, 46, 81–86]. These reports on improving heavy metal accumulation and tolerance of the plants indicate the potential applications of PCS on phytoremediation approaches.

Although PCS can be a molecular tool for phytoremediation of heavy metal-contaminated soils and waters, the overexpression of PCS promotes the catabolism of GSH, which also plays essential roles in redox reactions and heavy metal stress [87, 88]. If the metabolic pathways supplying GSH cannot maintain specific levels in the presence of highly expressed PCS, the consumption of GSH usually leads to changes in the GSH/GSSG ratio that exacerbate oxidative stress [62, 72]. The increased GSH demand driven by PC synthesis may also affect other metabolic pathways requiring GSH [89]. In these regards, the use of functional PCS with diminished catalytic activity could reduce the depletion of GSH, maintain redox homeostasis and supporting PC synthesis during exposure to heavy metals at the same time [62]. Indeed, the Arabidopsis and *Brassica juncea* transgenic lines expressing a partially deactivated AtPCS1 mutant, AtPCS1-Y186C, showed enhanced Cd^{2+} tolerance and higher GSH/GSSG ratio than the transgenic lines expressing wild-type AtPCS1 [62]. These results suggest that PC synthesis and redox homeostasis are both important for successful heavy metal resistance.

Besides the imbalance of cellular redox state, PCS overexpression could result in an unknown disruption in cellular metal homeostasis under heavy metal stress because PCS itself is a metalloenzyme and can bind a wide range of metal ions [90, 91]. Expressing synthetic genes encoding peptide analogs of PCs with a general structure of $Met(Glu\text{-}Cys)_nGly$ (n = 16–20) could be an alternative way to enhance the accumulation of heavy metals in the plants without the overexpression of PCS [92]. The Arabidopsis transgenic plants transformed with the artificial genes encoding these PC-like polypeptides resulted in hyperaccumulation of Cd^{2+} and $As^{3+/5+}$ in the plants [92]. However, the impact of accumulating synthetic PC-like polypeptides on the overall metal homeostasis is yet to be determined.

3.2 Phytochelatin synthase-involving pathway engineering for enhancing heavy metal accumulation

Pathway engineering involved in the co-expression of both GSH synthesis and PC synthesis pathways is another strategy to preserve the balance of GSH metabolism in the cells with constitutive PC synthesis [72, 74]. A kinetic model of GSH and phytochelatin synthesis in plants suggests that at least two enzymes, γ-glutamylcysteine synthetase (γ-ECS) and PCS, should be increased to enhance PC synthesis without depleting the GSH pool [89]. In fact, the effects of modified GSH/PC synthesis pathways have been tested in *Escherichia coli* and tobacco plants, respectively [93, 94]. In these experiments, the activities of SpPCS and two enzymes catalyzing the rate-limiting steps of GSH biosynthesis, including serine acetyltransferase (SAT) and γ-ECS, were enhanced (**Figure 1**) [7, 8, 93, 94]. The *E. coli* cells co-overexpressing these enzymes accumulated significantly higher concentrations of PCs and Cd^{2+}, while the single-gene expression in the PC synthesis pathway had limited effects [93]. These findings support the "gene stacking" approaches to enhancing heavy metal metabolism. Although the same strategy co-overexpressing these three genes in tobacco increased some classes of non-protein thiol, the Cd^{2+} accumulation in the transgenic plants did not change compared to the wild type [94]. These findings suggest that other mechanisms, in addition to the availability of precursors for PC synthesis, limit Cd accumulation in plants [94].

Overall, the genetic engineering approaches involved in manipulating PC synthesis have shown promising prospects for improving the performance of plants in the phytoremediation of heavy metals. However, there are also setbacks pointing at the complexity of the stress response induced by heavy metals [75]. Thus, while enhanced PC synthesis can contribute to the heavy metal chelating, other factors, such as the subsequent vacuolar sequestration or the delicate balance of GSH metabolic pathways under heavy metal stress, should be considered in order to achieve heavy metal tolerance.

4. The role of phytochelatin synthase in glutathione-*S*-conjugate metabolism

4.1 The involvement of phytochelatin synthase in the catabolism of glutathione derivates

PCS has a broad substrate selectivity and can use GS-derivates as substrates. For example, PCS isolated from plant species can accept *S*-alkylated GSH such as *S*-methyl-GS and *S*-hexyl-GS [6, 95] and large side residues like xenobiotic GS-conjugates (abbreviated as GS-conjugates) [17, 19, 20]. The bulky *S*-residues of GSH that can be converted to γ-Glu-Cys-conjugates by PCS include benzyl-, nitrophenyl-, phenylbenzyl-, uracil-, bimane-, and acetamido-fluorescein-groups [17, 19]. However, when PCS uses GS-derivates with these bulky *S*-linked side residues, it tends to transfer the γ-Glu-Cys-conjugate intermediate to a hydrogen group [17, 19, 20]. As a result, PCS processes the hydrolysis of GS-conjugates instead of their polymerization.

Besides its significant role in heavy metal detoxification, PCS also participates in the biodegradation of xenobiotic compounds because of its capability to process GS-conjugates [17–20]. Glutathione conjugation is a major pathway to inactivate xenobiotic compounds in plant cells [7]. Glutathione transferase (GST) detoxifies xenobiotics in the cytosol by transferring these compounds to GSH [10, 11, 96, 97]. These GS-conjugates enter vacuoles rapidly for sequestration and further degradation [12, 13]. In Arabidopsis, the transport of GS-conjugates for vacuolar sequestration

is facilitated by AtABCC1/AtMRP1 and AtABCC2/AtMRP2, which also transfer PC-metal complexes into vacuoles [12, 13, 22, 24]. Because of the high efficiency of this sequestration mechanism, the subsequent catabolism of GS-conjugates is presumably processed in the vacuoles [18]. First, vacuolar γ-glutamyl-transpeptidase (GGT) initializes the degradation of GS-conjugates by removing the γ-Glu-residue from the GS-conjugates to form Cys-Gly-conjugates [14, 15], and then, carboxypeptidase cleaves the Gly residue and results in the accumulation of the Cys-conjugates [16]. Alternatively, the GS-conjugate degradation can be initiated by PCS when vacuolar sequestration is not an available route [17–20]. The pathways of GS-conjugate metabolism are summarized in **Figure 1**.

4.2 Phytochelatin synthase may participate in initiating the first step of glutathione-*S*-conjugates degradation in the cytosol

Monochlorobimane (MCB) is widely used as a model xenobiotic for Arabidopsis to study the catabolism of GS conjugates [14, 15, 17–20, 98]. The bimane-labeled thiols can be analyzed by high performance liquid chromatography [99]. In addition, the fluorescent GS-bimane can be directly monitored *in situ*, which indicates the compartmentation and the turnover of GS-conjugates [15, 18, 20]. Data have shown that AtPCS1 initiates the first step of GS-bimane degradation in cytosol by removing the Gly residue and providing substrates for the vacuolar GGT [17, 19–21] (**Figure 1**). This detour could be a functionally alternative route to detoxify xenobiotics when the major pathway is blocked [17–20].

The direct evidence showing the involvement of PCS in GS-conjugate metabolism is the defects in the turnover of GS-bimane shown in the Arabidopsis AtPCS1-deficient mutants [21, 47]. The AtPCS1-deficient mutant, *ΔPCS1*, and the AtPCS1/AtPCS2 double-deficient mutant, *ΔPCS*, are impaired in the degradation of GS-bimane to γ-Glu-Cys-bimane [19, 20]. Blum et al. (2007) report that in the absence of Cd^{2+}, the abundance of the γ-Glu-Cys-bimane in both *ΔPCS1* and *ΔPCS* mutants was significantly reduced compared to the wild type after the plants were challenged by the xenobiotic bimane [19]. Moreover, the induction of γ-Glu-Cys-bimane was not observed in AtPCS1-deficient lines in the plants treated with Cd^{2+}, which resulted in a > 10-fold lower γ-Glu-Cys-bimane accumulation compared with the wild type grown in the same conditions [19]. The GS-baimane concentration could be rescued by transfecting AtPCS1 cDNA into PCS-deficient protoplasts, suggesting that this process is indeed PCS-dependent [19]. The inhibited γ-Glu-Cys-bimane accumulation in the mutant lines indicates that AtPCS1 efficiently catalyzes GS-conjugates in the presence of Cd^{2+} [19, 20].

Although the GS-bimane conversion is altered in the AtPCS1-deficient mutants, the GS-bimane in these mutants still can be degraded through the major detoxification pathway in the vacuoles [18–20]. Besides, the overall turnover of GS-bimane in the mutants is only slightly affected without blocking the vacuolar transport pathway [18–20]. These findings underline that the vacuolar GGT-initiated GS-conjugates degradation is the major pathway among two compensatory routes responsible for the turnover of the xenobiotics [18].

In plant cells, both the cytosolic PCS and the vacuolar carboxypeptidase can catalyze the formation of γ-Glu-Cys-bimane [16–19]. However, the vacuolar carboxypeptidase tends to catalyze the Cys-Gly-conjugates following the cleavage of γ-Glu-residue initiated by GGT [15]. In this regard, PCS is supposed to be the primary component responsible for the γ-Glu-Cys-bimane formation observed in the process of GS-conjugate conversion. Another example showing the importance of PCS in the initiation of the cytosolic xenobiotic compound is the metabolism of the herbicide safener fenclorim [100]. Fenclorim enhances GST activity in Arabidopsis

and is subsequently degraded via the GS-conjugation pathway [97, 100]. In the Arabidopsis suspension cells, GS-fenclorim was sequentially processed to γ-Glu-Cys-fenclorim and Cys-fenclorim, suggesting that deglycination is the initial step to the catabolism of fenclorim [21, 100]. However, more evidence is needed to confirm the direct involvement of PCS in this process.

4.3 The glutathione-*S*-conjugate conversion via phytochelatin synthase is metal-dependent

The presence of metal ions is a critical requirement for PCS-dependent catalysis of GS-bimane [17–20]. Intriguingly, the efficiency of GS-bimane hydrolysis activated by different metal ions is separate from that of metal-stimulated PC synthesis [17]. For example, the PC formation of AtPCS1 activated by Cd^{2+} is usually 2–5 times more efficient than the PC synthesis rate measured in the presence of $Cu^{+/2+}$ [4, 6, 53]. On the other hand, AtPCS1 could catalyze the deglycination of GS-bimane 60% more efficiently in $Cu^{+/2+}$ solutions than in the presence of Cd^{2+} [17]. It was suggested that *in vivo* AtPCS1 is a Cu-containing metalloenzyme in unstressed conditions, and consequently, the Cu-bound PCS favors the catalysis of GS-conjugate over PC synthesis in the normal growth conditions [17]. Evidence supporting this hypothesis is that in Arabidopsis, the deglycination of GS-bimane was PCS-dependent in the absence of heavy metals [19]. However, considering AtPCS1 binds Cu^{2+} only at a low affinity [6, 21], and the majority of cytosolic Cu is usually associated with Cu chaperons [101], it is possible that the concentration of free cytosolic Cu ions is not sufficient to fully activate PCS for the catalysis of GS-conjugates.

5. Can phytochelatin synthase play a part in the phytoremediation of combined pollutions?

With global industrialization and the development of modern cropping systems, massive amounts of toxic substances such as pesticides, heavy metals and inorganic fertilizers have been released into the environment and caused massive pollutions [97, 102]. Inevitably, the combined contaminations have damaged the ecosystems and become global issues [103, 104].

In the case of soil pollution, the primary sources of heavy metals include pesticides, fertilizers, mining, industrial processing and wastewater [102, 105]. One example of combined pollutants is glyphosate-based herbicides, which are highly toxic to the environment yet are the most-used pesticides in the world [106]. Heavy metals such as As, Ni, and Pb, which activate the catalytic activity of PCS, can be found as contaminants in many commercial glyphosate-based herbicides [3, 6, 106]. Interestingly, the *in vivo* chronic regulatory experiments showed that the toxicity of these herbicides might come from the heavy metals included in formulants instead of the active ingredients [106]. These findings suggest that heavy metal toxicity may occur in the biological materials used in the phytoremediation of xenobiotic compounds. Thus, heavy metal detoxification mechanisms in phytoremediation plants also need to be considered to improve their performance in co-contaminated soils and groundwaters.

GSH and its derivates are widely involved in plant development and stress response, and GSH itself serves as a hub for the mechanisms of heavy metal detoxification, xenobiotics biodegradation and oxidative stress response [7, 9]. Because PCS is a key enzyme in GSH metabolism, it is not surprising that PCS should be involved in both heavy metal stress and the turnover of xenobiotics. Other critical enzymes in the GSH metabolic pathway such as GST have been used in combating

multiple stresses, including heavy metal and xenobiotics degradation, and biotic stress [107]. However, the role of PCS is primarily emphasized in heavy metal stress response despite its contribution to the degradation of GS-metabolites and innate immunity. Based on the knowledge about the diverse functions of PCS, it is worth exploring whether PCS can be a useful tool in enhancing the tolerance and performance of the plants challenged by combined stresses.

6. Conclusion

Both heavy metals and pesticides significantly arrest plant growth and development. The co-contamination of soils by both heavy metals and pesticides has raised concerns regarding crop safety and productivity, and is therefore crucial to remediate. Phytoremediation presents the advantages of high efficiency, low cost, and sustainability. Thus, it has been one of the most common strategies for the remediation of polluted soils. This chapter summarizes the critical role of PCS in heavy metal detoxification and the involvement of PCS in GS-conjugate degradation. In the presence of heavy metals, PCS catalyzes the synthesis of PCs and the initiation of GS-conjugate metabolism. Despite a large body of literature illustrating the function of PCS in heavy metal resistance, there has been less emphasis on the participation of PCS in the detoxification of xenobiotic compounds and its potential application in biodegradation. Given that PCS has diverse functions in different types of stress, this chapter discusses the potential inclusion of PCS to achieve phytoremediation for combined pollutions.

The key question related to PCS overexpression in plant materials for phytoremediation is how GSH homeostasis can be balanced. Although pathway engineering enhancing GSH metabolism and PCS activity seems a promising approach, the consequences of manipulating these pathways may not directly lead to improving the performance of plants exposed to stress, due to the complexity of the cellular GSH network. Thus, the challenge for the future is not only to characterize the involvement of PCS in stress responses but also to broaden our knowledge in PCS as a factor that regulates GSH status and cellular redox homeostasis.

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