Mini-review

INvolvement of Glutathione in Abscisic Acid Signaling and Methyl Jasmonate Signaling in Guard Cells

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Stomata are formed by pairs of guard cells, which control the gaseous exchange and transpirational water loss in plants. The opening and closure of stomata are regulated by the integration of numerous environmental signals and endogenous hormonal stimuli. In response to drought stress, plants synthesize a hormone, abscisic acid (ABA), which triggers the signal transduction in the guard cells and induces stomatal closure that prevents water loss by transpiration. Methyl jasmonate (MeJA) is a phytohormone that regulates various physiological processes and mediates plant defense responses. Similar to ABA, MeJA plays a role in the induction of stomatal closure. Glutathione (GSH; \(\gamma\)-glutamylcysteinyl glycine) is an abundant, ubiquitous, and non-enzymatic antioxidant that has significant functions in the growth, development, defense systems, signaling, and gene expression in plants. In recent years, many studies have shown that GSH is involved in the ABA- and MeJA-induced stomatal closure. In this study, we outline the involvement of GSH in the stomatal closure and discuss how GSH regulates ABA signaling and MeJA signaling in the guard cells.

Key Words: abscisic acid, glutathione, guard cells, methyl jasmonate, reactive oxygen species

1. INTRODUCTION

Stomata are tiny pores on the leaf epidermis of higher plants that serve as a pathway both for the influx of CO\(_2\) into the plants from the atmosphere for optimal photosynthesis and for the transpirational water loss\textsuperscript{1}. A stomatal aperture is controlled by a pair of specialized guard cells surrounding the stoma. The guard cells are able to sense and integrate multiple environmental and endogenous signals\textsuperscript{2-3}. Abscisic acid (ABA) controls many physiological processes, such as seed maturation, dormancy, and vegetative growth, in plants and helps plants to withstand various adverse environmental conditions such as cold, salinity, and drought\textsuperscript{1-3}. Under a drought condition, plants synthesize ABA that induces stomatal closure for suppressing transpirational water loss.

Methyl jasmonate (MeJA) regulates various developmental processes and mediates defense responses in plants. It has been reported that MeJA also induces stomatal closure in many plant species similar to ABA\textsuperscript{4}. Glutathione (GSH) is the most abundant non-protein thiol compound in plants and is involved in many physiological and cellular processes\textsuperscript{5-6}. Environmental stresses induce oxidative damage by the excessive production of reactive oxygen species (ROS) in plants. Glutathione regulates the ROS-related and redox-sensitive signal transduction factors under normal and stressful conditions in plants. Several pieces of evidence have suggested that the ABA and MeJA signaling pathways in guard cells involve redox regulation and are regulated by GSH. Reactive oxygen species and cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)\(_{cyt}\)) function as signaling components.
both in the ABA and MeJA signal transduction in the guard cells\(^{7,8}\). In this review, we focused on the involvement of GSH in ABA- and MeJA-induced stomatal closure to find out how GSH regulates the ABA- and MeJA-induced signaling in the guard cells.

2. BIOSYNTHESIS OF GLUTATHIONE

Glutathione is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine. Enzymatically, the biosynthesis of GSH from its constituent amino acids occurs through a two-step reaction at the expense of two molecules of ATP\(^9\). In the first step, γ-glutamylcysteine is formed from L-glutamate and L-cysteine, catalyzed by γ-glutamylcysteine synthetase (γ-ECS). The second step is the synthesis of GSH through the addition of glycine to the C-terminal of γ-glutamylcysteine that is catalyzed by GSH synthetase (GSHT). In Arabidopsis thaliana, γ-ECS is found in the plastids and GSHT is found in both the plastids and cytosol\(^10\). Thus, the first step of glutathione synthesis occurs in the plastids, and then γ-ECS is exported from the plastids to the cytosol where it serves as a precursor to the GSH biosynthesis\(^11\). From the cytosol, the synthesized GSH can be transferred into other organelles to meet metabolic requirements. Under stress conditions, different biochemical reactions take place within different cellular compartments and the GSH is continuously oxidized to a glutathione disulfide (GSSG) form that is again converted to GSH by NADPH-dependent glutathione reductase (GR). This thiol-disulphide interaction is considered as the most fundamental and recognized function of GSH. For sustainable growth and development, plants generally maintain higher levels of reduced glutathione than those of oxidized glutathione. The concentration of the reduced GSH and the ratio between GSH and GSSG play a substantial role in the redox regulations of cellular processes\(^12\).

3. FUNCTION OF GSH IN PLANT GROWTH AND DEVELOPMENT

Glutathione takes part in many metabolic functions in plants, such as detoxification of heavy metals\(^13\) and methylglyoxal, a cytotoxic compound that is a byproduct of glycolysis under stress conditions\(^14\). Apart from the detoxification, GSH participates in the reduction of adenosine 5’-phosphosulphate as the first reductive step of sulfate assimilation\(^15\). Another important function of GSH is scavenging ROS\(^9,16\). Different ROS are generated as by-products through normal aerobic metabolism in plants. There are two ways by which GSH is involved in the detoxification of ROS, i.e., the ascorbate-glutathione cycle\(^9\) and the glutathione peroxide cycle\(^6\). Increased levels of endogenous GSH allow the efficient scavenging of ROS and thereby provide the plant cells with relief from oxidative stress experienced during abiotic stresses\(^12\).

4. REGULATION OF STOMATAL SIGNALING IN GUARD CELLS

(1) Depletion of GSH in guard cells during ABA- and MeJA-induced stomatal closure

Many investigations have been done to determine the roles of GSH in ABA as well as MeJA signaling in the guard cells. The stomatal movement of a glutathione-deficient mutant of Arabidopsis, cad2–1 (cadmium-sensitive), reveals how GSH is involved in the ABA- and MeJA-induced stomatal closure\(^8,17\). The cad2–1 mutant is deficient in the first enzyme of GSH biosynthesis, the γ-ECS\(^8\). Both the ABA and MeJA application narrow the stomatal apertures, and the stomatal apertures of cad2-1 plants are significantly narrower than those of the wild-type plants regardless of the ABA and MeJA treatment, indicating that the enhanced stomatal closure of cad2-1 is the result of genetic depletion of GSH in the guard cells\(^8,17\).

The glutathione content in guard cells can be observed by monochlorobimane (MCB) staining. The MCB reacts with intracellular GSH to form fluorescent GSH S-bimane (GSB) in the guard cells\(^8,17\). Both the ABA and MeJA decrease the amount of GSH in the guard cells of the wild-type plants and of the cad2-1 mutant while the cad2-1 mutant accumulates lower levels of GSH than the wild-type does\(^8,17\), suggesting that stomatal closure induced by ABA and MeJA is accompanied by depletion of the GSH content in the guard cells.

A GSH-decreasing chemical, CDNB (1-chloro-2,4-dinitrobenzene) depletes the GSH content in the guard cells by forming a conjugate with GSH\(^19\). Treatment with CDNB significantly enhances the extent of the ABA- and MeJA-induced stomatal closure in Arabidopsis, indicating that chemical depletion of GSH in the guard cells enhances the ABA- and MeJA-induced stomatal closure\(^8,17\).

The glutathione monoethyl ester (GSHmee) can permeate cell membranes and is hydrolyzed by cytosolic esterases to release free intracellular GSH\(^20\). Treatment with GSHmee significantly increases the intracellular GSH content in the cad2-1 guard cells and complements the stomatal phenotype.
of the mutant\textsuperscript{17}. The stomatal aperture in the cad2-1 mutant after treatment with GSHmee is not significantly different from that in the wild type in the presence or absence of either ABA or MeJA\textsuperscript{8)(17}. These results further confirm that the enhanced stomatal closure in the cad2-1 mutant is due to GSH depletion.

Another mutant, chlorina1-1 (ch1-1), accumulates less GSH due to the impairment of the light-harvesting protein in photosystem II, which also shows enhanced stomatal closure accompanied by decreasing GSH in the guard cells\textsuperscript{8)(17). Treatment with GSHmee increases the stomatal apertures of ch1-1 regardless of ABA or MeJA application, indicating that supplementation with intracellular GSH suppresses stomatal closure\textsuperscript{8)(17)(21). Taken together, these results suggest that intracellular GSH is closely involved in the ABA- and MeJA-induced stomatal closure, and that both ABA and MeJA cause stomatal closure with the depletion of GSH level in the guard cells.}

(2) Negative regulation of ABA and MeJA signaling by GSH

In guard cells, ROS act as second messengers in the ABA and MeJA signaling. Both ABA and MeJA induce stomatal closure along with the ROS production, which is mediated by NAD(P)H oxidases, and the produced ROS activate the \( I_{Ca} \) channels\textsuperscript{22)(23)(24}. Regardless of the ABA treatment, the depletion of GSH by CDNB does not significantly affect the ROS production\textsuperscript{8). In addition, the increasing intracellular GSH by GSHmee treatment does not decrease the ROS accumulation\textsuperscript{8}. These results indicated that ABA-induced ROS production is not modulated by the amount of GSH in the guard cells\textsuperscript{8). MeJA elicits ROS production in the wild-type guard cells as well as in the cad2-1 mutant guard cells\textsuperscript{17). No significant difference is observed in the ROS production between the wild-type guard cells and the cad2-1 mutant guard cells. Moreover, the increasing intracellular GSH by GSHmee treatment shows no significant effect on the MeJA-induced ROS accumulation in the cad2-1 mutant guard cells\textsuperscript{17). Therefore, similar to the ABA-induced ROS production, the MeJA-induced ROS production is not affected by the amount of GSH in the guard cells. Both ABA and MeJA elevate the cytosolic free calcium concentration ([Ca\(^{2+}\))\textsubscript{cyt}] and regulate the elevation of [Ca\(^{2+}\))\textsubscript{cyt}] in the guard cells\textsuperscript{24)(25)(26). It has been reported that ABA-induced and MeJA-induced stomatal closure is also accompanied by cytosolic alkalinization in the guard cells, which is closely related to the cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\))\textsubscript{cyt}] elevation\textsuperscript{7). In the cad2-1 guard cells, ABA activates the \( I_{Ca} \) currents regardless of the presence of the cytosolic GSH\textsuperscript{8}. Treatment with GSH does not affect the \( I_{Ca} \) currents and treatment with GSHmee does not affect the ABA-induced [Ca\(^{2+}\))\textsubscript{cyt}] elevation in the guard cells\textsuperscript{8). Similarly, other studies of the MeJA signaling showed that GSH increased by the GSHmee treatment does not affect the MeJA-induced ROS production or [Ca\(^{2+}\))\textsubscript{cyt}] elevation via Ca\(^{2+}\) channel activation\textsuperscript{21} and that neither depletion by CDNB nor addition of GSH by GSHmee affects the cytosolic alkalinization by MeJA\textsuperscript{17). Taken together, these results suggest that GSH functions as a negative factor of both the ABA and MeJA signaling, but does not play upstream of the activation of the \( I_{Ca} \) currents and [Ca\(^{2+}\))\textsubscript{cyt}] elevation in either the ABA or MeJA signal cascade (Fig. 1).}

![Simple model of ABA and MeJA signaling in the guard cells. Glutathione functions as a negative factor of both the ABA and MeJA signaling, and acts downstream of the activation of the \( I_{Ca} \) currents and [Ca\(^{2+}\))\textsubscript{cyt}] elevation in either the ABA or MeJA signal cascade in the guard cells.](image)

5. CONCLUSION

Abscisic acid-induced stomatal closure as well as MeJA-induced stomatal closure is accompanied by decreasing GSH in the guard cells and that decreased GSH sensitizes the guard cells to ABA and MeJA during the ABA-induced and MeJA-induced stomatal closure in Arabidopsis. Exogenous application of GSH has the potential to eliminate oxidative conditions caused by ABA and MeJA. However, the interactions between GSH and other molecules, such as phytohormones, NO, H\(_2\)O\(_2\), and Ca\(^{2+}\) during signaling are still poorly understood. Therefore, future studies are needed to clearly elucidate the involvement of GSH in the signaling pathways in order to protect plants against various environmental stressed conditions.

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