学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Studies on Stomatal Response to Sulfur Dioxide in Arabidopsis
（シロイヌナズナにおける二酸化硫黄に対する気孔応答の研究）

学位論文の要旨 Abstract of Thesis

Stoma, which consists of a pair of guard cells in the epidermis of vascular plants, ingeniously controls transpirational water loss and carbon dioxide (CO_2) uptake under biotic and abiotic stresses in the environment. Besides, stomata are also the first entrance of gaseous pollutants into the plant body. Environmental-polluting gases, such as ozone (O_3), sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) are known to close stomata. Stomatal closure against hazardous gases is postulated as one of the protection mechanisms of plants, yet it is still unproven until today. SO_2 is one of the major airborne pollutants known to impacts natural vegetation and crop production. Although the effects of SO_2 on plants have been extensively studied since 1848, its effects on stomata remained concealed with only a handful of reports on stomatal response against SO_2 in the past century, which are inconsistent and ambiguous. Here, I investigated the mechanism of SO_2-induced stomatal closure; identified the responsible chemical species in SO_2 aqueous solution that is responsible for the stomatal closure induction; molecular biologically examined stomatal response to SO_2 using Arabidopsis mutants; explored the involvement of cell death in guard cells during exposure to SO_2, and the roles of hormone signaling and other signaling pathways in SO_2-induced stomatal closure.

The mechanism of SO_2 diffusion into the plant body and its effects on plant metabolism have long been established. Nevertheless, the action of SO_2 in inducing stomatal closure is unknown. Three chemical species (H_2SO_3, HSO_3^-, and SO_3^{2-}) are formed when SO_2 gas dissolved into water depending on the pH of the solution, yet the chemical species that is responsible for stomatal closure induction remained concealed. I examined the involvement of each chemical species in stomatal closure induction by observation on stomatal response to a wide range of concentrations of these chemical species. It was identified that H_2SO_3 renders stomatal closure in a concentration-dependent manner. This result suggests that H_2SO_3 is the only SO_2-derived chemical species that closes stomata

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in the presence of SO_2.

Although several harmful gases were reported to induce stomatal closure, the molecular mechanisms of the closure have not been well investigated except for O_3, of which *SLOW ANION CHANNEL-ASSOCIATED 1/OZONE-SENSITIVE-1* (*SLAC1/OZS1*) and *OPEN STOMATA 1 (OST1/SNRK2.6/SRK2E)* had been identified as the critical factors in regulating O_3-induced stomatal closure. The aforementioned molecular factors are also recognized to be involved in CO_2-induced stomatal closure, in addition to *RESPIRATORY BURST OXIDASE HOMOLOG*s (*RBOH*s). Considering the partially redundant phenotypes in the O_3- and CO_2-insentive stomata mutants and the structural similarity among CO_2, O_3, and SO_2, which comprised of three atoms with two oxygen atoms on both ends, I speculated that plants share parts of the regulators in response to gaseous stimuli through stomatal closure. Here, I tried to identify if SO_2-induced stomatal closure is regulated by the same molecular factors which regulate O_3- and CO_2-induced closure, using Arabidopsis CO_2- and O_3-insensitve stomata mutants. Studies in SO_2-induced chlorophyll degradation and stomatal closure induction did not demonstrate significant differences in the response of the mutants from wild type. Mutants utilized are all demonstrating open-stomata phenotype. SO_2-induced stomatal closure in these mutants suggesting that other closure mechanism that are not regulated by *SLAC1*, *OST1* and *RBOH*s is involved. This study advocates that SO_2-induced stomatal closure is a distinctive event, which is different from O_3- and CO_2-induced closure.

Phytohormones and gasotransmitters, such as NO and H_2S, are also postulated to be involved in stomatal regulations. Abscisic acid (ABA) production has been implicated in SO_2-induced stomatal closure. Another potential example of the participation of a hormone in gas-induced stomatal closure is jasmonic acid (JA) in CO_2 response. Moreover, an antecedent pharmacological study in stomatal response utilizing *Ipomoea batatas* reported the roles of NO and H_2S production in response to SO_2. To investigate the involvement of phytohormones in SO_2-induced stomatal closure, I quantified the contents of phytohormones in H_2SO_3-treated leaves. As oppose to previous studies, no correlation was identified in between the levels of major defense hormones (JA and salicylic acid, SA) and the kinetic of SO_2-stomatal closure. This further emphasizes that SO_2-induced stomatal closure is of a different mechanism from CO_2 responses. The involvement of ABA was excluded with no significant increase observed in the ABA levels in leaves treated with H_2SO_3. Furthermore, no significant differences were observed in stomatal response to SO_2 in solutions with and without scavengers of H_2S and NO excluding the involvement of NO and H_2S signaling pathways in SO_2-induced stomatal closure, at least for Arabidopsis.

SO_2-induced stomatal closure has been postulated to be due to cytoplasmic acidification that inhibits K^+ influx of the membrane, accumulation of ABA and the involvement of H_2S and NO signaling pathways, in *Vicia faba*, *I. batatas* and *Pisum sativum*. As oppose to these claims,

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I hypothesized that it was instead due to the damage of vital functional organelles which causes the closure of stomata. I speculated that H_2SO_3 kills stomatal guard cells after reaching to the cytosolic liquid forming SO_3^{2-} and HSO_3^- ions, leading to a decrease in cytosolic pH with the release of additional H^+ ions, which then causing the stomatal closure. Decreasing viability rate of stomatal guard cells was observed in leaves treated with increasing concentrations of H_2SO_3 where ~100% mortality rate was attained at higher H_2SO_3 concentrations, indicating H_2SO_3 kills stomatal guard cells in a concentration-dependent manner. Here, I also investigated if SO_2 induces stomatal opening at low concentrations as previously reported in *V. faba*. An apparent two-phasic distribution of stomatal aperture width was observed in stomatal response to low concentration of H_2SO_3, treated under the light. This suggests that a portion of stomata had started to close (due to the death of guard cells) while another portion of them had opened wider (due to the release of constraint from surrounding epidermal pavement cells which have lost turgor). SO_2-induced cell death in the guard cells was identified to be non-apoptotic, indicating that SO_2-induced stomatal closure is not a biological process to protect foliage against the entrance of harmful gas, but it is solely a physicochemical process resulted from SO_2 distress.

In conclusion, I have identified that the responsible SO_2-derived chemical species for stomatal closure induction is H_2SO_3. To provide new insight into the potential common mechanisms in stress avoidance response of stomata against hazardous gases, I have examined the stomatal response of O_3- and CO_2-insensitive stomata mutants to SO_2. It is suggested that the molecular mechanism that induces stomatal closure against SO_2 is different from O_3 and CO_2. The involvement of phytohormones and gasotransmitters NO and H_2S in SO_2-induced stomatal closure. Besides, SO_2 has been reported to induced stomatal opening at low concentrations in addition to closure induction at high concentrations. Here, my findings suggest that SO_2 promotes stomatal opening in the light, while provoking cell death in the guard cells at the same time. I also concluded that SO_2-induced stomatal closure is highly correlated to non-apoptotic cell death in the guard cells.