

Hydrogen sulfide: the shutter button of stomata in plants

Zhuping Jin & Yanxi Pei*

School of Life Science, Shanxi University, Taiyuan 030006, China

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In the past few decades, Hydrogen sulfide (H₂S) has been newly witnessed as a third gasotransmitter with similar biological functions to its two previously identified counterparts nitric oxide (NO) and carbon monoxide (CO), after being recognized as a toxic gas with an unpleasant rotten-egg smell for hundreds of years. Following the recognition of its great pharmacological potential in mammalian (Mani et al., 2015), H₂S has also been revealed as a crucial player involved in the regulation of physiological processes in normal plants. It has also been found to be a critical messenger in plant defense responses against a variety of biotic and abiotic stresses at physiological concentrations. The H₂S-synthesizing enzymes, located in various subcellular compartments such as the cytoplasm, mitochondria and chloroplast, are more abundant in plants than in animals and show a spatial-temporal expression pattern (Jin and Pei, 2015).

Eyes are the windows to the soul. Stomata, pores on the leaf surface, which are the “eyes” of plants, have been demonstrated to actively engage in various cellular aspects under different changing environmental conditions. Stomata open or close in response to environmental stimuli such as atmospheric humidity, light, atmospheric CO₂ concentration and plant hormones. It has been reported that H₂S responds to drought stress by inducing stomatal closure in *Arabidopsis thaliana*, *Vicia faba* and *Impatiens walleriana* (Jin et al., 2011; Jin et al., 2013). The initial research was based on pharmacological experiments by applying exogenous H₂S donor (NaHS and GYY4137) (Jin et al., 2011).

Later on *AtLCD* (L-cysteine desulhydrase) mutants were used to provide genetic evidence to support the physiological role of endogenous H₂S on stomatal movement (Jin et al., 2013). Similarly, H₂S was also confirmed to participate in ethylene and NO-induced stomatal closure. Analysis of GUS activity from the transgenic plants harboring different fragments of the promoter indicated that the key section of *AtDCD1* (D-cysteine desulhydrase gene) promoter in response to ethylene came from –697 to –408 bp whereas the key section in response to drought stress was from –90 to –1 bp (Liu et al., 2011; Hou et al., 2016). Interestingly, another research group reported that exogenous H₂S could induce stomatal opening by reducing the accumulation of NO in guard cells of *A. thaliana* and a crop plant, *Capsicum annuum* (Lisjak et al., 2010). Thus, the effect of H₂S on stomatal movement remains a controversial topic. The discrepancy might be simply due to the different experimental systems and distinct features of plant materials. Under any circumstances, the consequence caused by H₂S is to ultimately enhance the tolerance of plants under abiotic stress. Induction of stomatal closure is to reduce the moisture loss under drought stress, while the purpose of stomatal opening might decrease the photorespiration and enhance photosynthesis. With this question, it is intriguing to further investigate the mechanism of stomatal movement regulated by H₂S.

Regulation of the turgor of the two guard cells that surround the pore is essential to the control of stomatal aperture. Their volume and turgor are reduced by efflux of anions and potassium ions (K⁺) and by gluconeogenic conversion of malate into starch, which causes membrane depolarization and stom-

*Corresponding author (email: peiyanxi@sxu.edu.cn)

atal closure. Stomatal opening, in contrast, is mediated by the increase in K^+ , anion and malate concentrations in the guard cells. It is therefore noted that ion channels in cell membranes have become an important way of material exchange between organisms and the surroundings, which facilitate the identification of genes encoding specific ion channels and transporters. Recent studies focused on H_2S regulating ion channels at different levels. Initially, expression of Calcium (Ca^{2+}) channel and outward-rectifying K^+ channel coding genes were found decreased with the reduction of endogenous H_2S , and expression of inward-rectifying K^+ increased conversely (Jin et al., 2013). Subsequently, H_2S was found to promote the genes expression, the phosphorylation level of PM H^+ -ATPase and Na^+/H^+ antiporter protein level, which regulates plasma membrane Na^+/H^+ antiporter system in response to salt stress. Meanwhile, H_2S was found to inhibit K^+ efflux and maintain relatively high K^+/Na^+ ratio in guard cells of alfalfa to enhance salt stress tolerance. In tobacco, H_2S selectively inhibiting K^+ influx channels played a significant role in the ABA-induced stomatal closure. It is worth noting that Ca^{2+} participated in the regulation of K^+ influx by ABA, but not involved in the process of H_2S -induced stomatal closure (Papanatsiou et al., 2015). Taken all together, the results suggested that H_2S might be a critical link in stomatal regulation by its interaction with ABA via ion channels in the signaling transduction network. Consistently, WRKY transcription factors participating in ABA signaling pathway regulated the H_2S signaling pathway in plants under cadmium stress (Liu et al., 2015).

To further exploit the mechanism of H_2S on stomatal movement, the patch-clamp technique has been applied to test S-type anion channels in the plasma membrane of guard cells recently. The direct evidence of H_2S -activating S-type anion channel currents in *Arabidopsis* guard cells was provided. In addition, the activation of anion currents by H_2S was mediated by SLAC1 channels and cytosolic free Ca^{2+} . At the same time, protein kinase OST1 functions in H_2S -induced activated SLAC1 channel currents (Wang et al., 2016). These electrophysiological results accurately showed H_2S -induced stomatal closure through numerous ions flow. At this point, the pattern of H_2S regulating stomatal closure seems to have a conclusion.

In summary, H_2S acting as a switch, adjusts to shut down stomata when plants encounter adverse environment. To find out the exact mechanism of stomatal movement regulated by

H_2S , the further investigation will reveal the secret of ion channels *in vivo*, as well as the speed and direction of ion flow through other emerging technologies such as NMT (noninvasive micro-test technique).

Meanwhile, S-sulfhydration of proteins modified by H_2S in mammals attracted the attention of researchers. H_2S might inhibit L-type calcium currents depending on the sulfhydryl group in rat cardiomyocytes. Presumably, S-sulfhydration of the ion channels in guard cells of plant mediated by H_2S will turn out to be a focal point in the near future to unravel the mysteries of stoma shutter button.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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