Nitric oxide and hydrogen sulfide share regulatory functions in higher plant events

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Abstract: Nitric oxide (NO) and hydrogen sulfide (H₂S) are two molecules that share signaling properties in plant and animal cells. NO and H₂S originate two families of derived molecules designated reactive nitrogen and sulfur species (RNS and RSS, respectively). These molecules are responsible for certain protein regulatory processes through posttranslational modifications (PTMs), being the most remarkable S-nitrosation and persulfidation, which affect the thiol group of cysteine residues. NO and H₂S can also exert regulatory functions due to their interaction through the iron present in proteins that contain heme groups or iron-sulfur clusters, as reported mainly in animal cells. However, the available information in plant cells is still very limited thus far. In higher plants, NO and H₂S are involved in a myriad of physiological events from seed germination to fruit ripening, but also the mechanism of response to biotic and abiotic stress conditions. This viewpoint manuscript highlights the functional regulatory parallelism of these two molecules which also interact with the metabolism of reactive oxygen species (ROS) in plant cells.

Brief Historical Perspective

Nitric oxide (NO) and hydrogen sulfide (H₂S) are two gaseous molecules that were initially considered dangerous because they were associated with some detrimental effects on animal and plant cells. However, this perspective underwent a drastic change of mind when it was found that these molecules were endogenously generated in animal cells (Kolluru et al., 2013). There was a gap of about 10 years between the initial research works that described the signaling functions of either NO or H₂S in living organisms. Accordingly, key research on NO, published in 1987 (Palmer et al., 1987), and on H₂S in 1996 (Abe and Kimura, 1996) in animal systems provided the first pieces of evidence showing that these molecules exerted diverse signaling roles in the cardiovascular and nervous systems, respectively. Years later, plant biologists also found that these molecules were also endogenously generated in plant cells where they are involved in almost all the stages of plant development including seed germination, root development, plant growth, stomata movement, senescence, flowering and fruit ripening (Lesher et al., 1998; Lamattina et al., 2003; Simontacchi et al., 2004; Corpas et al., 2004; Corpas et al., 2006; Corpas et al., 2008; Zhou et al., 2018; Chen et al., 2019; González-Gordo et al., 2019; Mukherjee and Corpas, 2020; Zuccarelli et al., 2021). And both compounds were also linked to the mechanisms of response against adverse environmental conditions triggered by either abiotic or biotic agents (Corpas, 2019; Kharbech et al., 2020; Iqbal et al., 2021). Fig. 1 illustrates the key functions in which NO and H₂S have been shown to participate in higher plants.

Chemistry and Biochemistry of NO and H₂S

Although NO and H₂S are very simple molecules, their (bio)chemistry is more complex than it could be thought (Stamler et al., 1992; McCleverty, 2004; Hughes, 2008; Kabil and Banerjee, 2010; Filipovic et al., 2018; González-Gordo et al., 2020). NO is a colourless gas that belongs to the free radical-type molecules because it has an unpaired electron in the π orbital of the nitrogen atom, what is usually indicated with a dot in the chemical formula (‘NO). Some of the NO and H₂S physical and chemical properties are: (i) Solubility of NO is 1.9 mM in aqueous solutions at 1 atm pressure, whereas the solubility of H₂S is 100 mM at the same pressure; (ii) Their in vivo lifetime is relatively short,
NO and H2S can interfere with the function of the target molecules.

Likewise, NO and H2S can also interact with iron containing-proteins, where the metal is present as either heme group or as part of the iron-sulfur cluster. Thus, there are multiple examples in higher plants where either NO and H2S, or both, can modulate, through their interaction with the cysteine thiol groups, the functions of proteins such as cytochrome c oxidase, catalase, Fe-superoxide dismutase, ascorbate peroxidase, ferredoxin(Fd)-NADP reductase, glutaredoxin, Fd-dependent glutathione:2-oxoglutarate aminotransferase (Fd-GOGAT) or phytooglobin, (Ramirez et al., 2011; Aroca et al., 2017; Bahmani et al., 2019; Palma et al., 2020; Niu et al., 2019; Corpas et al., 2021). These proteins are involved in essential plant processes including photosynthesis, respiration, antioxidant system, nitrogen and sulfur assimilation, which remarks the physiological relevance of these signaling molecules. However, in higher plants, the available information about the direct interaction of NO and H2S with the iron side of protein is still scarce. In addition to reactions that can originate the respective families of NO and H2S with the iron side of protein is still scarce. In addition to reactions that can originate the respective families of NO and H2S, NO or H2S can react with thiol groups from peptides and proteins affecting the function of the target molecules. NO and H2S are lipophilic molecules and they can diffuse across cell membranes; and, (iv) Both molecules can react with thiol groups from peptides and proteins affecting the function of the target molecules.

Ascorbate Peroxidase (APX) in Plant Cells: A Case Study of NO and H2S Target

In-plant systems, the number of identified proteins that undergo PTMs mediated by either NO or H2S has progressively increased thanks to the efforts of many researchers focused on this biochemical area (Lindermayr et al., 2005; Tanou et al., 2009; Fares et al., 2011; Begara-Morales et al., 2013; Kato et al., 2013; Chen et al., 2014; Aroca et al., 2015; Liu et al., 2019). Moreover, the analyses of these modified proteins have revealed that many of them can be the simultaneous target of both PTMs and, by *in vitro* assays, it has been also proven the relevance of these two regulatory molecules to modulate the biological activity of the affected proteins (Muñoz-Vargas et al., 2018, 2020; Palma et al., 2020; Corpas et al., 2021). Among the different plant proteomic studies focused on the identification of the potential targets of PTMs mediated by either NO or H2S, it has been found that ascorbate peroxidase (APX) is one of these shared targets.

S-nitrosation and Persulfidation: Two Protein PTMs that Exert Redox Control of Thiol Groups

The amino acid cysteine (Cys) can play relevant roles in proteins such as a structural function through disulfide bonds, but it could also have implications on redox reactions by means of its thiol group (–SH). Thus, depending on the conditions surrounding the thiol group in the protein, Cys can be found in its anionic form, designated as thiolate (RS–), which is a stronger nucleophilic agent than its protonated form (Netto et al., 2007). Fig. 2A depicts the different oxidation states of sulfur which range from thiol (–2) to sulfonic acid (+4). Among these states, NO or H2S can interact with the thiolate form through either S-nitrosation or persulfidation (Fig. 2B), also known previously as protein S-nitrosylation and S-sulfhydration, respectively (Aroca et al., 2018; Wolhuter et al., 2018; Corpas et al., 2019, Corpas et al., 2021). For that reason, Cys is considered as a redox switch in the protein metabolism because it is the main target of these two PTMs, and this could affect significantly the biological activity of the corresponding protein, either positively or negatively.

**FIGURE 1.** Main processes where both nitric oxide (NO) and hydrogen sulfide (H2S) are involved in higher plants.

**FIGURE 2.** (A) Oxidation states of sulfur (S) in proteins from thiol (–2) to sulfonic acid (+4) forms. Under cellular oxidant conditions, the oxidation from sulfenic acid becomes irreversible. The numbers in parenthesis represent the different oxidation states of S in the protein. (B) Protein thiol modifications mediated by NO (S-nitrosation) and H2S (persulfidation).
APX is a key antioxidant enzyme that is part of the ascorbate-glutathione cycle, which is an essential system to modulate the mechanism of response against (a)biotic stress environmental conditions (Shigeoka et al., 2002; Asada, 2006; Maruta and Ishikawa, 2018). APX is a hemoprotein that controls the cellular level of hydrogen peroxide (H2O2) according to the following reaction:

\[ \text{L-ascorbate} + \text{H}_2\text{O}_2 \rightarrow \text{dehydroascorbate} + 2\text{H}_2\text{O} \]

This enzyme system is composed of different isozymes located in almost all subcellular compartments including cytosol, chloroplasts, mitochondria and peroxisomes (Asada, 1992; Yamaguchi et al., 1995; Bunkelmann and Trelease, 1996; Jiménez et al., 1998; Yoshimura et al., 1999; Maruta et al., 2016; Chin et al., 2019). This molecular and location diversity suggests the great relevance of APX in cell signaling under physiological and stressful conditions, which is consolidated by its regulation through both S-nitrosation and persulfidation, as indicated above. Furthermore, it was identified by mass spectrometric analyses that the Cys32 is the residue that underwent S-nitrosation and persulfidation (Begara-Morales et al., 2014; Yang et al., 2015; Aroca et al., 2015) and, in both cases, the APX activity was positively regulated. This mimicking biochemical regulation provides a clear connection between NO and H2S with the metabolism of reactive oxygen species (ROS) (Rodriguez-Ruiz et al., 2017), thus indicating the biochemical link among all these families of molecules.

Conclusions and Future Perspectives

The previous perception of NO and H2S as harmful molecules to plant cells has drastically changed and, nowadays, they are key signal molecules that regulate a myriad of biochemical and physiological processes. These two gases have also families of derived molecules designated as reactive nitrogen and sulfur species (RNS and RSS, respectively). They have a wide range of biochemical implications, being S-nitrosation and persulfidation two representative examples of their cellular relevance which compete molecularly to modulate protein function. This similarity is reinforced by the fact that they are able to mimic each other in the physiological and stressful conditions, which is consolidated by its regulation through both S-nitrosation and persulfidation (Begara-Morales et al., 2014; Yang et al., 2015; Aroca et al., 2015) and, in both cases, the APX activity was positively regulated. This mimicking biochemical regulation provides a clear connection between NO and H2S with the metabolism of reactive oxygen species (ROS) (Rodriguez-Ruiz et al., 2017), thus indicating the biochemical link among all these families of molecules.

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