Plant Signaling & Behavior 4:9, 878-879; September 2009; © 2009 Landes Bioscience

Ozone and nitric oxide interaction in *Arabidopsis thaliana*

A role for ethylene?

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Nitric oxide (NO) is involved together with reactive oxygen species (ROS) in the activation of various stress responses in plants. However, the biochemical mechanisms by which ROS and NO participate, and the potential interaction between these molecules are still unclear. Ozone (O_3) can be used as a tool to elicit ROS-activated stress responses and to activate cell death in plant leaves. We have recently shown that O_3 induced a rapid accumulation of NO in Arabidopsis leaves and at late time points NO production coincided with the formation of hypersensitive response like lesions.[1] Experiments using O_3 and the NO-donor SNP alone or in combination indicated that both molecules are capable of activating a large set of stress related genes. In combined treatment, NO attenuated O_3-induction of salicylic acid (SA) biosynthetic and signaling genes, and reduced SA accumulation. In addition, NO can elevate the levels of ethylene in several mutants. Thus, NO is a modifier of ROS signaling.

Key words: cell death, reactive oxygen species, ozone, nitric oxide, ethylene

Abbreviations: NO, nitric oxide; ROS, reactive oxygen species; O_3, ozone; HR, hypersensitive response; SA, salicylic acid; SNP, sodium nitroprusside

Submitted: 07/03/09

Accepted: 07/03/09

Previously published online: www.landesbioscience.com/journals/psb/article/9428

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Addendum to: Ahlfors R, Brosché M, Kollist H, Kangasjärvi J. Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. Plant J 2009; 58:1–12; PMID: 19054359; DOI: 10.1111/j.1365-313X.2008.03756.x.

Ozone (O_3) is an air pollutant which in sensitive plants causes a reduction in photosynthesis and growth, ultimately leading to decreased growth and crop yields. O_3 induces the production of ROS by the cells affected.[2] These ROS include superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). The O_3-induced quick burst of ROS resembles the oxidative burst in the hypersensitive response (HR) in incompatible plant pathogen interactions.[3] The responses that follow the ROS burst include changes in the synthesis and accumulation of the plant stress hormones ethylene, salicylic acid (SA) and jasmonic acid and changes in gene expression.[4,5] Eventually, the production of ROS can lead to HR-like cell death.[2,5]

In addition to ROS and hormones, nitric oxide (NO) has a prominent role in the defense against pathogens.[6] Previous studies have shown that NO is quickly produced after pathogen attack and is involved in cell signaling during HR in plants.[7,8] NO efficiently induces changes in gene expression[9] suggesting that it has an active role in modifying plant stress responses. An important feature of NO is its lipophilic nature which enables its diffusion across plant membranes. NO can alter post-translational signaling by nitrosylation and/or direct binding to active centers of proteins, collectively making NO an effective signaling molecule.[6]

Recently we have shown that NO can modify cell death responses, signaling and gene expression in O_3-exposed Arabidopsis plants.[1] O_3 induced NO production in a transient manner and production of NO occurred mainly in the area of O_3-induced microlesions. Transcript profiling indicated a role for NO in attenuation of certain classes of O_3 induced genes, many of which were related to SA biosynthesis or SA signaling. Furthermore, using mutants with altered levels of NO production we demonstrated that functional NO production is required for proper plant O_3 responses. One of the earliest plant responses to O_3 is ethylene production.[2] A few studies indicate that NO and ethylene could interact in regulating stress responses, for example NO treatments induce production of ethylene

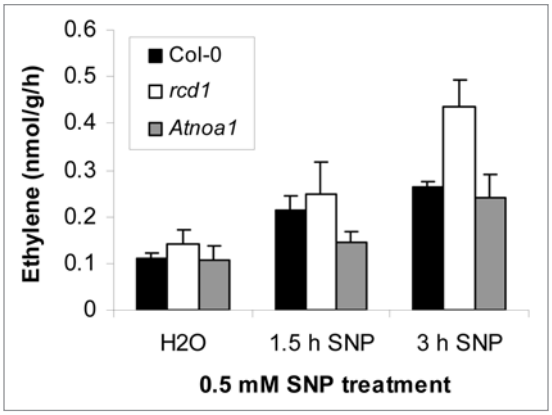

Figure 1. NO activates ethylene biosynthesis. 0.5 mM SNP treatment of Col-0, *rcd1* and *Atnoa1* lead to increase in ethylene formation.

in Arabidopsis and tobacco,[10,11] and NO and ethylene are proposed to act together to regulate some O_3 induced genes, exemplified by alternative oxidase *AOX*.[11]

To further dissect the interaction between NO and O_3 we observed that both SNP (sodium nitroprusside) and O_3 treatment of Col-0 increased expression of the ethylene biosynthesis related genes, *ACS6* and *ACC oxidase*.[1] O_3 induced *ACS6* expression correlates with ethylene formation.[12] To confirm that SNP induce ethylene production we measured ethylene production in Col-0 and two mutants, *rcd1* (NO overproducer) and *Atnoa1/rif1* (reduced NO-production). SNP treatment increased ethylene production, particularly in the *rcd1* mutant (**Fig. 1**). Interestingly, combined SNP + O_3 treatment led to attenuation of *ACS6* expression[1] indicating that one role of NO during O_3 could be to modulate ethylene responses.

Both SA and ethylene are regulators of ROS induced cell death.[2,4] We propose that NO has a role in O_3 induced signaling by modifying SA and ethylene levels and gene expression levels. Ethylene is known to be involved in lesion propagation and the elevated ethylene levels in all genotypes tested, especially in the O_3 sensitive *rcd1* (**Fig. 1**) suggests that NO indeed has a role in modifying hormone biosynthesis and accumulation.

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