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Regulation of Ascorbate Contents by Jasmonate-Mediated Signaling Pathway in *Arabidopsis* during Ozone Exposure

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K e y w o r d s : *Arabidopsis thaliana*, ethylene, jasmonate, ozone, salicylic acid, ascorbate.

S u m m a r y

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Jasmonates, composed of jasmonic acid (JA) and methyl jasmonate (MeJA), are widely distributed signaling compounds in plants. Jasmonate-mediated signaling suppresses ozone-induced ethylene biosynthesis as well as cellular injury since an ozone-sensitive *Arabidopsis* mutant, *oji1*, showed increased ozone-induced ethylene production and reduced sensitivity to MeJA (KANNA & al. 2003). Although *oji1* plants had higher JA contents than the wild-type (Ws-2) plants during exposure to 0.2ppm ozone, microarray analysis revealed decreased expression of genes for enzymes in JA biosynthesis in this mutant. On the other hand, salicylic acid contents and expression of salicylic-acid inducible and biosynthetic genes in *oji1* plants were similar to those in the wild-type plants until 12 h after the beginning of ozone exposure. In spite of foliar injury, ascorbate contents

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increased in *Arabidopsis* by ozone exposure. While *oji1* plants revealed lower ascorbate contents than the wild-type plants, exogenous MeJA suppressed the increase in ascorbate contents in the wild-type plants at 6 h after the beginning of ozone exposure. These results imply that jasmonate-mediated signaling is involved in the regulation of the defense system in the surviving cells under stress conditions.

Introduction

Plants are severely damaged by ozone which is the major component of photochemical oxidants. Ozone induces production of phytohormones such as ethylene, salicylic acid (SA) and jasmonates in plants via reactive oxygen species as signal transduction materials: ethylene and SA promote injury to ozone-exposed plants whereas jasmonates have defensive roles. In addition to the role of each hormone, cross-talk between hormone-mediated signaling pathways is thought to regulate the plant's response to ozone (TAMAOKI & al. 2003a). Ozone-induced ethylene production was suppressed by treatment with methyl jasmonate (MeJA), one of jasmonates, in *Arabidopsis* and an ozone-sensitive mutant showed an enhanced level of ozone-induced ethylene, which was not suppressed by MeJA treatment (KANNA & al. 2003). Since this mutant has reduced sensitivity to MeJA estimated by inhibition of root growth (*ozone-sensitive jasmonate-semi-insensitive 1*, *oji1*), the jasmonate-mediated signaling pathway putatively via OJI1 is supposed to include signal transduction involved in inhibition of cell elongation and suppression of stress ethylene production, and not to function normally in this mutant.

While hormone-mediated signaling pathways are thought to be involved in programmed cell death (OVERMYER & al. 2003), the antioxidative system, which consists of antioxidant enzymes and redox materials such as ascorbate, plays important roles in prevention of cells from direct oxidation by reactive oxygen species. It has recently been indicated that the antioxidative system is involved in a hormone-mediated signaling network in plants (CONKLIN & BARTH 2004).

We analysed the relation between the antioxidative system and hormone-mediated signaling using an ozone-sensitive jasmonate-semi-insensitive *Arabidopsis* mutant, *oji1*, to identify the mechanisms for plant responses to environmental stresses. The results suggested the possibility of regulation of ascorbate level by jasmonate-mediated signaling under stress conditions caused by ozone.

Material and Methods

Plants of *Arabidopsis thaliana* L. ecotype Wassilewskija-2 (Ws-2) and a derivative mutant *oji1* were grown at 25 °C under 14-h light (100 µmol photosynthetic photon flux density (PPFD) $m^{-2}s^{-1}$) in a growth chamber for 2 weeks and exposed to 0.2 ppm ozone at 25 °C under continuous light (350 µmol PPFD $m^{-2}s^{-1}$) as described (KANNA & al. 2003).

Micoroarray analysis was conducted using mRNA from Ws-2 and *oji1* at 0, 3, 6, 12 and 24 h after the onset of ozone exposure. mRNA was labelled by Cy3 (0 h) or Cy5 (3, 6, 12 and 24 h) using CyScribe First-Strand cDNA Labelling Kit (Amersham Bioscience, Piscataway, NJ, USA) and hybridized with Agilent *Arabidopsis* 2 oligo DNA microarray kit (Agilent Technologies, Palo Alto, CA, USA) at 60 °C for 17 h, then the chips were washed at room temperature for 10 min and

at 4 °C for 5 min.

RNA gel blotting was performed as described (KANNA & al. 2003). Salicylic acid content was determined using approximately 100 mg of *Arabidopsis* seedlings as described (OGAWA & al. 2004). Ascorbate was determined using 1 to 6 plants of *Arabidopsis*.

Arabidopsis seedlings were sprayed with 0, 10 or 100 µM of methyl jasmonate and incubated in a plastic case covered with plastic cling film at 25 °C for 14 h in darkness before the ozone exposure.

Results and Discussion

In spite of higher JA contents in *oji1* plants than in wild-type plants during exposure to 0.2 ppm ozone (KANNA & al. 2003), a microarray analysis demonstrated decreased expression of genes for enzymes in JA biosynthesis in this mutant (Table 1). Relative expression to 0 h of lipoxygenase 2, allene oxide synthase, allene oxide cyclase and 12-oxophytodienoate reductase 3 was lower in *oji1* plants than in the wild-type plants throughout ozone exposure for 24 h. Hence, increased JA accumulation in *oji1* is not supposed to be a result of enhancement of JA biosynthesis, but probably because of inhibition of its degradation and/or metabolism due to deficiency in jasmonate-mediated signaling which includes a pathway via OJI1.

Table 1. Changes in gene expression during ozone exposure in Ws-2 (left) and in *oji1* (right) plants. Values were obtained by a microarray analysis as relative values (-fold) to the intensity of 0 h. DHAR; dehydroascorbate reductase, GLDH; L-galactono-1,4-lactone dehydrogenase, MDAR; monodehydroascorbate reductase. Localization in DHAR and MDAR were based on CONKLIN & BARTH 2004.

	3 h		6 h		12 h		24 h	
Lipoxygenase 2 (AT3G45140.1)	1.6	0.5	2.7	1.0	1.6	1.4	4.9	1.3
Allene oxide synthase (AT5G42650.1)	2.4	1.8	2.6	1.6	1.1	1.1	2.1	1.0
Allene oxide cyclase (AT1G13280.1)	1.8	1.1	1.2	1.2	1.8	1.5	1.9	1.3
12-oxophytodienoate reductase 3 (AT2G06050.1)	17.8	9.3	10.6	8.5	4.7	3.5	5.6	3.0
Phenylalanine ammonia lyase 1 (AT2G37040.1)	1.2	1.4	0.6	0.7	0.9	0.9	2.9	0.9
Glutathione S-transferase (AT1G02920.1)	28.9	9.2	27.2	10.8	7.4	4.2	6.7	3.9
GDP-mannose pyrophosphorylase (AT2G39770.1)	1.6	1.0	1.9	1.8	1.6	1.4	2.5	1.4
GLDH (AT3G47930.1)	0.8	0.9	0.8	1.1	1.0	1.0	0.9	0.9
DHAR, unknown localization (AT1G19550.1)	1.5	1.1	1.7	1.2	1.0	0.9	0.8	1.0
DHAR, cytoplasm(AT1G19570.1)	2.8	1.1	2.4	1.2	2.2	1.0	4.1	0.9
DHAR, cytoplasm/membrane (AT1G75270.1)	2.3	2.6	2.0	2.0	2.4	2.9	2.3	2.7
DHAR, plastids (AT5G16710.1)	0.9	0.6	0.8	0.5	0.4	0.4	0.7	0.4
MDAR, plastids, mitochondria(AT1G63940.1)	1.0	0.6	1.3	0.7	0.9	0.8	1.3	0.7
MDAR, mitochondria (AT3G27820.1)	0.8	0.5	0.9	0.6	0.7	0.8	0.9	0.7
MDAR, cytoplasm (AT3G52880.1)	1.1	1.0	0.9	1.1	1.0	1.1	1.0	1.0
MDAR, cytoplasm (AT5G03630.1)	8.1	6.3	10.3	9.9	8.2	7.3	5.2	6.8

On the other hand, SA contents from 3 sets of measurements until 6 h after the beginning of ozone exposure (data not shown) and expression of a SA inducible gene, *PR1*, analyzed by RNA gel blotting during a 12-h ozone exposure (data not

shown) did not show a significant difference between the *oji1* and wild-type plants. A microarray analysis also revealed that relative expression to 0 h of a SA biosynthetic gene, phenylalanine ammonia lyase 1, in *oji1* plants was similar to those in wild-type plants until 12 h after the beginning of ozone exposure (Table 1). Thus, jasmonate-mediated signaling via OJI1 is thought to have little influence on SA biosynthesis or its signaling at least in the early stage of ozone-induced injury.

Deficient jasmonate-mediated signaling via OJI1 seemed to affect gene expression of enzymes involved in the antioxidative system. In *oji1* plants, ozone-induced gene expression was reduced in a glutathione S-transferase, GDP-mannose pyrophosphorylase, a key enzyme of ascorbate biosynthesis, and most of ascorbate-recycling enzymes; dehydroascorbate reductases (DHAR) and monodehydroascorbate reductases (MDAR) (Table 1). These results indicate that not only the response of defense genes, but also both biosynthesis and recycling of ascorbate may be affected in *oji1*, although gene expression of another ascorbate biosynthetic enzyme, L-galactono-1,4-lactone dehydrogenese (Table1, GLDH), was not induced by ozone in Ws-2 nor affected in *oji1*. While *oji1* plants showed lower or similar expression in 7 of 8 isogenes for ascorbate-recycling enzymes, the expression of only one DHAR gene (AT1G75270.1) was minimally higher in *oji1* plants than in wild-type plants. This might possibly be complementary regulation of whole-cell activity of recycling of ascorbate by a DHAR which may not be regulated by jasmonate-mediated signaling via OJI1.

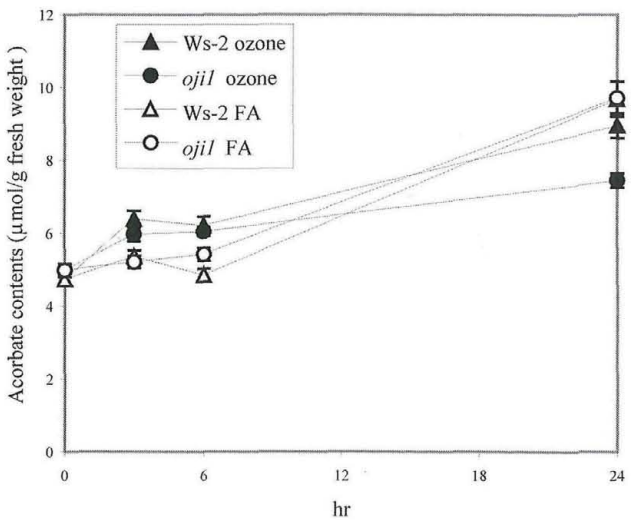

Fig. 1. Ascorbate contents during 0.2 ppm ozone exposure in *Arabidopsis*. Vertical bars are standard errors. N=9. FA, fresh air.

In *Arabidopsis*, ascorbate contents started to increase at 3 h after the onset of ozone exposure both in the wild-type and in *oji1* plants, and kept increasing dur-

ing the 24-h exposure in spite of foliar injury, although the effect of ozone on increase in ascorbate contents seemed to be restricted until 6 h (Fig. 1). This was in contrast to the decrease in ascorbate contents immediately after onset of ozone exposure in tobacco (AONO & al. 1997). At 3 h, *oji1* plants started to show visible foliar injury and the wild-type plants showed slight injury beginning at 6 h (data not shown). Taken together with ozone-induced expression of the gene for the key enzyme of ascorbate biosynthesis and ascorbate-recycling genes (Table 1), this indicates that ozone appears to induce biosynthesis and recycling of ascorbate in surviving cells of *Arabidopsis*, even in *oji1* severely injured by ozone. Ascorbate contents also increased in seedlings placed in the fresh air. The control conditions, i.e., the continuous light and higher light intensity than growth conditions, may increase ascorbate contents in *Arabidopsis* (TAMAOKI & al. 2003b) similarly both in the wild-type and *oji1*, through a signaling pathway possibly independent of jasmonate-mediated signaling via OJI1.

In ozone-exposed *oji1* plants, ascorbate contents were approximately 80% of that in the wild-type plants at 24 h after the onset of the exposure (Fig. 1). This is supposed to be mainly because of the decrease in number of vital cells by ozone-induced cell death. However, the possibility that lower ascorbate contents in *oji1* plants were due to a deficiency of jasmonate-mediated signaling which may possibly increase ascorbate contents cannot be entirely excluded (SASAKI-SEKIMOTO & al. 2004).

Pretreatment with MeJA suppressed the ozone-induced increase in ascorbate contents in a concentration-dependent manner in the wild-type plants at 6 h after the beginning of ozone exposure (Fig. 2 Ws-2). Ascorbate contents in *oji1* were not increased by ozone under these conditions and the effect of MeJA was not observed (Fig. 2 *oji1*). These results imply that jasmonate-mediated signaling is involved in the regulation of ascorbate contents, i.e., antioxidative defence system, in the surviving cells under ozone-induced stress conditions.

Jasmonate-mediated signaling pathway, however, seems to play opposite roles in regulation of ascorbate contents during ozone exposure. On one hand, exogenous MeJA may activate this signaling pathway and suppresses the increase in ascorbate contents in the wild-type plants. On the other hand, deficient jasmonate-mediated signaling in *oji1* failed to increase ascorbate contents during ozone exposure (Fig. 2). The jasmonate-mediated signaling pathway via OJI1 may have a role to up-regulate ascorbate contents in surviving cells upon onset of cell death in leaves, while this pathway, or another jasmonate-mediated signaling pathway not via OJI1, may also play a role in defence against cell death not by ascorbate. Presumably, since exogenous high concentrations of MeJA might induce other defence mechanisms than ascorbate biosynthesis or recycling, such as suppression of ethylene production to maintain cells against detrimental oxidative-stress conditions which promote cell death, ascorbate biosynthesis or recycling might not need to be activated in wild-type plants.

Foliar injury seemed to be slightly reduced in the MeJA-pretreated wild-type plants, but was not estimated quantitatively. No decrease in ascorbate contents by MeJA was observed in seedlings in the fresh air (Fig. 2). Until 6 h, the ascorbate

contents in ozone-exposed seedlings were lower in Fig. 2 than in Fig. 1, especially little ozone-induced increase was observed in *oji1* in Fig. 2. The pre-treatment condition, i.e., 14-h darkness in a wrapped plastic case after spraying of MeJA solution or deionized water as the control, may have affected the regulation of ascorbate in the early stage.

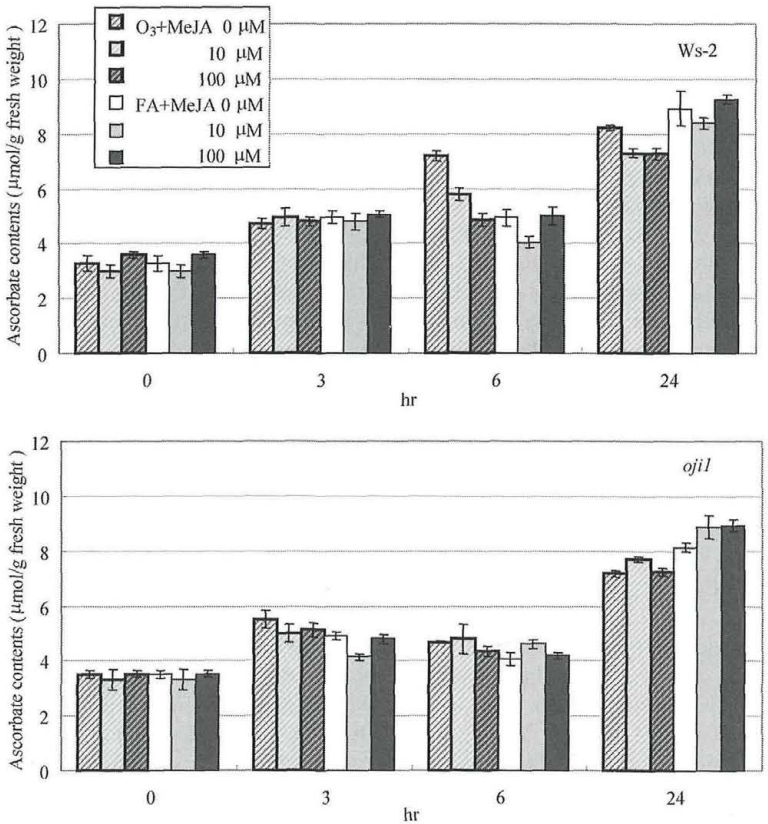

Fig. 2. Ascorbate contents in MeJA-pretreated *Arabidopsis* during 0.2 ppm ozone exposure. Vertical bars show standard errors. Triplicate experiments were conducted and the similar results were obtained. The result from one experiment is shown. N=3 for each value. FA, fresh air.

To clarify the jasmonate-mediated signaling network involved in the antioxidative mechanisms in plants under stress conditions, we need to investigate further the cell-based ascorbate contents, its redox state and localization of ascorbate and related enzymes in the surviving cells, as well as accurately analyse the gene expression responsive to ozone and other stresses.

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