Senescence-Promoting Effect of Arabidopside A

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Arabidopside A isolated from *Arabidopsis thaliana* is a rare oxylipin, containing 12-oxophytodienoic acid (OPDA) and dinor-oxophytodienoic acid (dn-OPDA) which are known as precursors of jasmonic acid (JA) and methyl jasmonate (MeJA). The senescence-promoting effect of arabidopside A was examined by an oat (*Avena sativa*) leaf assay under dark or continuous light condition. Arabidopside A promoted senescence of oat leaves, and the promoting activity was more effective than for JA and OPDA, and as strong as for MeJA, which was well known to be a senescence promoter. These results suggest that arabidopside A plays important roles in leaf senescence.

Key words: Arabidopside A, Senescence, Chlorophyll Degradation

Introduction

In our search for bioactive substances of *Arabidopsis thaliana*, we isolated a new oxylipin, arabidopside A [s*n*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(dinor-oxophytodienoyl)-monogalactosyl diglyceride], from the aerial parts of *A. thaliana* L. ecotype Col-0 (Hisamatsu *et al.*, 2003). Arabidopside A contains 12-oxophytodienoic acid (OPDA) and dinor-oxophytodienoic acid (dn-OPDA), which are known as precursors of jasmonic acid (JA) and methyl jasmonate (MeJA) (Baertschi *et al.*, 1988; Weber *et al.*, 1997). It is known that JA and MeJA play various important roles in plants (Hisamatsu *et al.*, 2004). For example, when MeJA was applied to oat leaf (*Avena sativa*) segments, chlorophyll (Chl) content decreased more than in segments without exogenous JA (Ueda and Kato, 1980). Recently it was clarified that chlorophyllase, which is the key enzyme involved in Chl degradation was induced by MeJA in *A. thaliana* (Tsuchiya *et al.*, 1999). It was suggested that arabidopside A may also act as a plant growth regulator of plant senescence, especially Chl degradation, since arabidopside A containing OPDA and dn-OPDA may be included in the chloroplast membrane (Weber, 2002). In this paper, we describe the senescence-promoting effect of arabidopside A under dark or light condition in oat leaf segments.

Materials and Methods

Spectrometric analysis

ESIMS and NMR spectra were taken on a platform LC (Waters) and AVANCE 500 (Bruker) instrument, respectively.

Isolation of arabidopside A

Seeds of *Arabidopsis thaliana* L. ecotype Col-0 (Brassicaceae) were purchased from LEHLE SEEDS (Round Rock, TX, USA). They were immersed in water for 2 d before sowing on rock wool (Rock fiber, NITTOBO, Tokyo, Japan). They have been cultured under continuous light (24 h light, ca. 4,000 lux) at 24 °C, until forming the flower bud. Aerial parts of *A. thaliana* (200 g) were extracted with MeOH (3 L), and the extracts evaporated to dryness *in vacuo* at 30 °C. Then the residue was partitioned between H_2O and EtOAc. The EtOAc-soluble portion was subjected to silica gel column chromatography ($CHCl_3$/MeOH 49:1 → 1:1, v/v). The $CHCl_3$/MeOH (19:1, v/v) elute was separated in 3 fractions of 40%, 75%, and 100% CH_3CN in water by Sep-Pak C18 cartridge chromatography (Waters). The 75% CH_3CN fraction was further separated by HPLC [Deverosil ODS HG-5, 10.0 × 250 mm (Nomura Chemical, Seto, Aichi, Japan) with CH_3CN/H_2O 3:1, v/v, at a flow rate of 2.5 mL/min, UV detection at 222 nm] to yield arabidopside A (t_R 10.0 min,

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7.4 mg), which was identified by NMR and ESIMS.

Arabidopside A: Colorless amorphous solid. – ^{1}H NMR (500 MHz, CD_3OD): δ = 7.96 (2H, dd, J = 5.9 and 1.9 Hz, H-8″, H-10″), 6.21 (2H, dd, J = 5.9 and 1.9 Hz, H-9″, H-11‴), 5.45 (4H, m, H-13″, H-14″, H-15‴, H-16‴), 5.30 (1H, m, H-2), 4.48 (1H, dd, J = 12.0 and 3.1 Hz, H-1a), 4.27 (1H, d, J = 7.6 Hz, H-1′), 4.25 (1H, dd, J = 12.0 and 6.5 Hz, H-1b), 4.03 (1H, dd, J = 11.0 and 5.5 Hz, H-3a), 3.87 (1H, d, J = 3.3 Hz, H-4′), 3.80 (1H, dd, J = 11.4 and 7.0 Hz, H-6′a), 3.78 (1H, dd, J = 11.0 and 5.7 Hz, H-3b), 3.76 (1H, dd, J = 11.4 and 5.3 Hz, H-6′b), 3.53 (2H, m, H-2′ and H-5′), 3.51 (1H, dd, J = 9.7 and 3.3 Hz, H-3′), 3.10 (2H, m, H-7″, H-9‴), 2.56 (2H, m, H-11″, H-13‴), 2.47 (2H, m, Ha-12″, Ha-14‴), 2.38 (4H, m, H_2-2‴, H_2-2⁗), 2.21 (2H, m, Hb-12″, Hb-14‴), 2.12 (4H, m, H_2-15″, H_2-17‴), 1.81 (2H, m, Ha-6″, Ha-8⁗), 1.66 (4H, m, H_2-3″, H_2-3‴), 1.38 (12H, m, H_2-4″, H_2-5″, H_2-4‴, H_2-5‴, H_2-6‴, H_2-7‴), 1.26 (2H, m, Hb-6″, Hb-8‴), 1.02 (6H, t, J = 7.5 Hz, H_3-16‴, H_3-18⁗). – ^{13}C NMR (125 MHz, CD_3OD): δ = 214.3 (C-10″, C-12‴), 175.6 (C-1″, C-1‴), 171.1 (C-8″, C-10‴), 134.6 (C-14″, C-16‴), 133.7 (C-9″, C-11‴), 129.0 (C-13″, C-15‴), 106.2 (C-1′), 77.6 (C-5′), 75.7 (C-3′), 73.2 (C-2′), 72.7 (C-2), 71.0 (C-4′), 69.5 (C-3), 64.8 (C-1), 63.3 (C-6′), 51.8 (C-11″, C-13‴), 46.6 (C-7″, C-9‴), 35.7 (C-2″, C-2‴), 32.6 (C-6″, C-8‴), 31.6 (C-6‴), 31.1 (C-4″, C-4‴), 30.9 (C-7‴), 29.3 (C-5″, C-5‴), 26.7 (C-3″, C-3‴), 25.8 (C-12″, C-14‴), 22.6 (C-15″, C-17‴) and 15.3 (C-16″, C-18‴). – ESIMS (pos.): m/z = 797 $[M+Na]^+$.

Bioassay

The oat leaf assay was used with modifications according to Ueda and Kato (1980, 1981). Oat (*Avena sativa* cv. zenshin, Sapporosaishuen Co., Hokkaido, Japan) seeds were germinated in vermiculite moistened with water and seedlings were grown under continuous white fluorescent light of about 4,000 lux at 24 °C for 7 d. Ten upper 3-cm leaf segments were placed on two layers of Toyo No. 1-filter paper moistened with 5 mL of test solutions in a Petri dish. The test compounds were dissolved in water containing 0.5 mg/mL of the surfactants Tween 80/sorbitan monooleate (7 : 3, w/w) with or without 0.1 μM kinetin. Leaf segments incubated with the same medium without test compounds were used as control. The Petri dishes were kept under dark condition at 24 °C, or placed under continuous white fluorescent light of about 4,000 lux at 30 °C for 4 d. The Petri dishes in the presence of kinetin were placed under continuous light condition. After observation of the leaf coloring, the leaf segments were extracted with 80% cold acetone and their Chl contents determined.

Jasmonic acid (JA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and methyl jasmonate (MeJA) was derived from JA by methylation with trimethylsilyldiazomethane (Hashimoto *et al.*, 1981). 12-Oxophytodienoic acid (OPDA) was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA).

Determination of chlorophyll

Each rephrase of leaf segments was homogenized in 10 mL of 80% cold acetone, and Chl content was estimated according to the method of Arnon (1949). The extract was centrifuged at 3,000 rpm for 5 min. The resulting pellet was resuspended in 5 mL of 80% cold acetone and centrifuged twice at the same speed for 5 min. The three supernatants were combined and adjusted to 30 mL in a volumetric flask. The absorbances of each extract at 645 and 663 nm were spectrophotometrically measured, and the Chl content was calculated. All experiments were repeated at least six times and the results were similar. These results were averaged and expressed as percentages of the Chl contents of oat leaf segments incubated without test compound.

Results and Discussion

The use of Sep-Pak C18 cartridge chromatography shortened drastically the time for HPLC separation. It was also become easy to separate arabidopside A and glycolipids, such as *sn*1-*O*-(octadecatrienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride.

In our senescence-promoting assay of oat leaf segments, the color of the leaf segments treated with arabidopside A and MeJA (Fig. 1) at 10^{-5} M turned almost yellow after incubation, which was different from that of leaf segments incubated without test compounds both under dark and light conditions. On the other hand, the yellowing of the leaf segments treated with JA and OPDA (Fig. 1) at 10^{-5} M was not promoted as compared to leaf segments treated with arabidopside A.

As shown in Fig. 2A, arabidopside A significantly promoted the decrease in Chl content of the

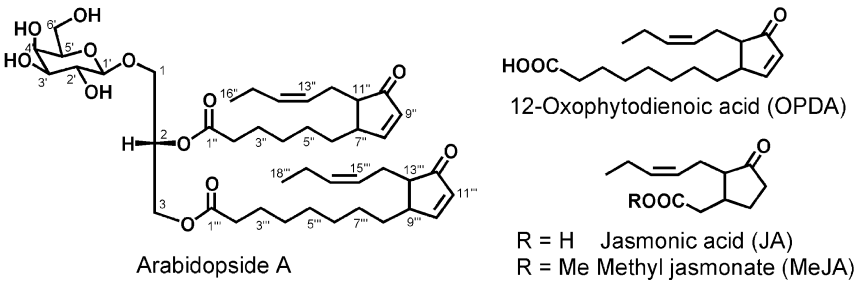

Fig. 1. Structures of arabidopside A, 12-oxophytodienoic acid (OPDA), jasmonic acid (JA), and methyl jasmonate (MeJA).

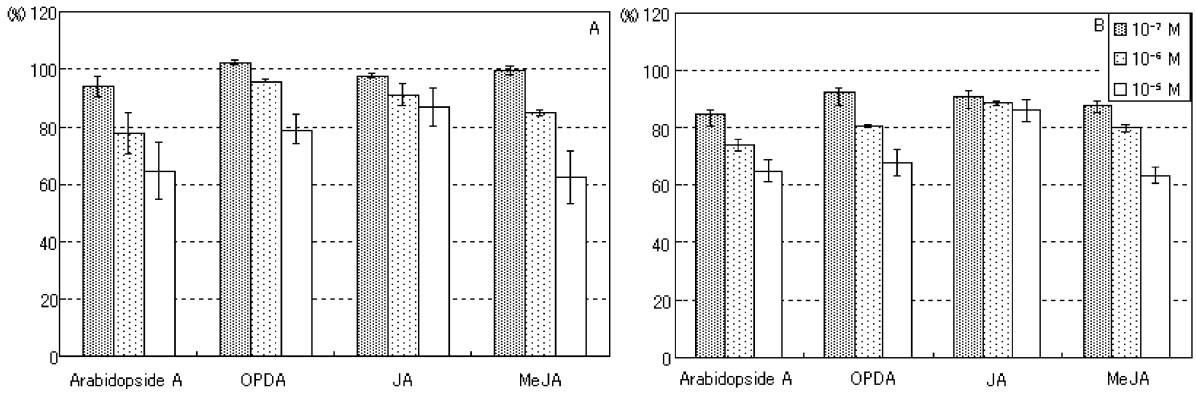

Fig. 2. Effects of arabidopside A, OPDA, JA, and MeJA on Chl content in oat leaf segments under dark (A) and continuous light (B). All solutions included 0.5 mg/mL of the surfactants Tween 80/sorbitan monooleate (7:3, w/w). Chl contents were determined 4 days after treatment and expressed as percentages of the Chl contents of oat leaf segments incubated without test compounds. Means ± SE of results from 6 replicates of 10 leaves.

leaf segment. Under dark condition, Chl content of the leaf segments treated with 10^{-5} M arabidopside A was about 35% of the initial Chl content. The effect of arabidopside A was as strong as that of MeJA, while JA and OPDA were less effective. Also under continuous light condition, arabidopside A promoted the loss of Chl content (Fig. 2B). Chl content of the leaf segments treated with arabidopside A and MeJA at 10^{-5} M decreased greatly, while that of the leaf segments without test compounds was only reduced gradually (data not shown). So it is assumed that arabidopside A is one of the substances promoting leaf segment senescence both in dark and in light.

Fig. 3 shows the Chl content of leaf segments incubated with kinetin under light condition. Degradation of Chl content without test compound was inhibited by kinetin. However, arabidopside A and MeJA promoted degradation of Chl content efficiently.

Since glycolipids containing OPDA/dn-OPDA have been isolated (Stelmach *et al.*, 2001; Ohashi *et al.*, 2005; Hisamatsu *et al.*, 2003, 2005), these

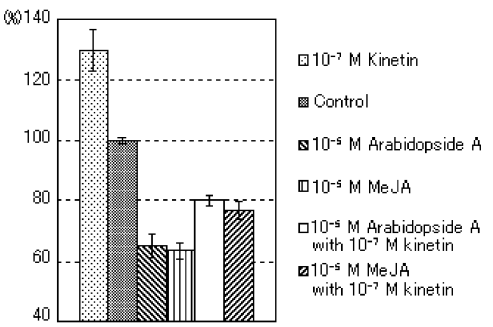

Fig. 3. Effects of arabidopside A and MeJA on Chl content in oat leaf segments under continuous light with 0.1 μM kinetin. All solutions included 0.5 mg/mL of the surfactants Tween 80/sorbitan monooleate (7:3, w/w). Chl contents were determined 4 days after treatment and expressed as percentages of the Chl content of oat leaf segments incubated without test compounds. Means ± SE of results from 6 replicates of 10 leaves.

compounds were rare, and their functions in plants were not again clear. However, some bioactivities and dynamics in stressed plants were reported. For example, *sn*1-*O*-(12-oxophytodienoyl)-monogalac-

tosyl glyceride isolated from *Ipomoea tricolor* showed stomatal opening activity (Ohashi *et al.*, 2005), and *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride was increased in wounded *A. thaliana* C24 leaves (Stelmach *et al.*, 2001). Recently, we also reported that arabidopsides A, B, and D inhibited the root growth of cress seedlings (Hisamatsu *et al.*, 2005). In addition to these reports, the promoting senescence effect of arabidopside A was observed for the first time. Therefore glycolipids containing OPDA/dn-OPDA may play important roles in various bioactivities of plants.

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