Journal of Plant Biology, March 2000, 43(1) : 56-59 **SHORT COMMUNICATION**

The Effects of Two Abscisic Acid Analogues, WL19224 and WL19377, on Stomatal Closure

Joon-Sang Lee*
Department of Life Science, Sangji University, Wonju 220-702, Korea

The effect on stomatal closure by ABA and its analogues, WL19224 and WL19377 was investigated. The rate of closure showed a sigmoid curve when various concentrations of ABA were applied. A concentration of 10^{-9} M ABA was the threshold for stomatal closure; maximal closure occurred at higher concentrations (from 10^{-6} M to 10^{-3} M). Use of the analogue WL19224 resulted in similar closure responses. However, ABA was more effective at lower concentrations. For example, at 10^{-3} M of either WL19224 and ABA, stomata closed to 2.2 μm and about 3 μm, respectively. In contrast, applications of the ABA analogue WL19377 had no effect on stomatal closure. In fact, at concentrations of WL19377 higher than 10^{-4} M, stomata were stimulated to open, to about 10% of their initial size. Likewise, applications of WL19377 along with ABA, inhibited ABA-induced stomatal closure. This inhibition was linearly related to the concentrations of the compounds applied. In conclusion, the structural requirements for biological activity of ABA and its analogues cannot be considered individually, but must be assessed for their roles as part of an entire functional group. Although compounds may have similar structures, their ability to control certain physiological activities may be quite different.

Keywords: ABA analogues, Abscisic acid, Stomatal closure

ABA is a ubiquitous plant hormone in vascular plants, and more than 109 ABA analogues have been reported (Davies, 1987). Because of its main role in moderating a plant's response to freezing, salt, or water stress, ABA has been characterized as a stress hormone. Under drought conditions, leaf-ABA concentrations can increase up to 40 times, which is the most dramatic change in concentration reported for any hormone in response to an environmental signal. ABA is very effective in causing stomatal closure; its accumulation in stressed leaves helps to reduce water loss by transpiration under water-stress conditions (Raschke, 1987).

MacRobbie (1992) has presented a plausible explanation for ABA-induced closure. The scheme involves Ca^{2+} influx through a nonselective cation channel as the first event, thereby producing depolarization and an increase in cytoplasmic Ca^{2+}. This may then be supplemented by a release of Ca^{2+} from internal stores, triggered by inositol 1,4,5-trisphosphate produced by activation of phospholipase C. The resultant increase in cytoplasmic Ca^{2+} causes deactivation of the inward K^+ channel, and activation of the Ca^{2+}-dependent anion channel. However, some other trigger is required to explain the outward K^+ channel.

The chemical structure of ABA determines its physiological activity. ABA has an asymmetric carbon atom at the 1' position in the ring, which results in the (+) and (–), or S and R, respectively. The naturally occurring enantiomer of ABA in plants and in the fungus *Cercospora rosicola* is S-(+). Commercially available synthetic ABA is a mixture of approximately equal amounts of (+) and (–) forms. Cummins and Sondheimer (1973) compared the effects of R- and S-ABA on the closure of stomata in detached *Hordeum vulgare* leaves. The effectiveness of S-ABA was much greater than that of R-ABA in closing stomata. Side-chain isomerism of ABA also relates its effect on stomatal closure. Conversion of the 2-cis, 4-trans-pentadienoic side chain of ABA to the all-trans isomer has been shown to dramatically reduce the activity of ABA in a variety of bioassays (Milborrow, 1966; Oritani and Yamashita, 1970a; Sondheimer and Walton, 1970; Kriedemann et al., 1972). Although changes in the length of the side chain of ABA have caused drastic reductions in activity (Mousseron-Canet et al., 1970; Oritani and Yamashita, 1970b; Sondheimer and Walton, 1970), ABA analogues have also been reported with altered side-chain lengths that are active in several assays.

In this study, the ABA analogues WL 19377 and WL 19224 (Fig. 1) were tested to determine their effects on stomatal closure, and to understand what parts of the structures of the analogues determined their physiological activity.

*Corresponding author; fax +82-371-730-0403
e-mail jslee@chiak.sangji.ac.kr

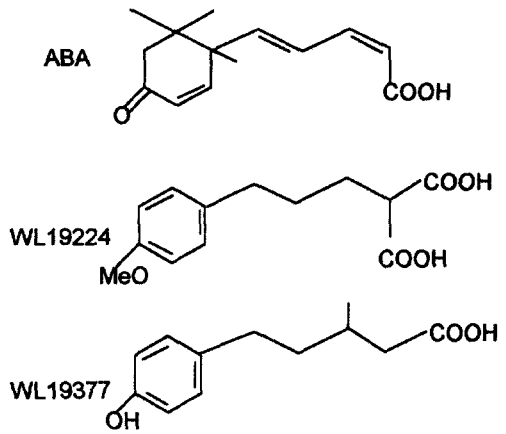

Figure 1. The structures of ABA and its analogues, WL19224 (3-(4-methoxyphenyl) propylpropaneclioic acid) and WL19377 (5-(4-hydroxyphenyl)-3-methylpentanoic acid).

MATERIALS AND METHODS

Plant Materials and Cultures

Commelina communis L. was grown from seed in John Innes No. 2 potting compost in a heated greenhouse (minimum temperature 20°C). Supplementary light provided a photoperiod of 16 h and a photon flux density (PFD) of 100 μ mole quanta $m^{-2}s^{-1}$. Five-weeks-old plants were transferred to a controlled-environment room, at 25 ± 1°C and with a 16-h photoperiod (PFD of 100 μ mole quanta $m^{-2}s^{-1}$). The plants were kept under these conditions for three days before use to ensure their adaptation after transfer). At all stages of development the plants were periodically watered to avoid water stress.

Stomatal Aperture Measurements

Epidermal strips were obtained from fully expanded leaves, following the method of Lee and Bowling (1992). The strips were cut into 5 × 10-mm pieces and placed in 5-cm Petri dishes containing 10 mM MES buffer (2-[N-morpholino] ethan sulphonic acid). The buffer was adjusted to pH 6.2 with KOH in which 50 mM KCl was dissolved. The dishes were incubated in a water bath for 3 h at 25 ± 1°C, under a PFD of 160 μ mole quanta $m^{-2}s^{-1}$. Carbon dioxide-free air was obtained by passing room air through a cylinder of soda lime and a 2 M KOH solution. When the stomata were fully open, either ABA (Sigma Chemical Co., Poole, U.K) or one of its analogues, WL 19224 and WL 19377, was added to the medium, and the epidermal strips were then incubated for another hour. Stomatal apertures were measured with a calibrated ocular micrometer disc under a microscope (400x; Leitz Labovert, Wurtzlar, Germany).

RESULTS AND DISCUSSION

Figure 2 shows a typical dose response of guard cells to applied ABA. Maximum closure rates were found at a concentration of (+/–) cis-trans ABA (10^{-6} M). Therefore, this concentration was used in all subsequent experiments involving the ABA analogues.

The effect of stomatal closure by WL19224, whether as a singular treatment or in combination

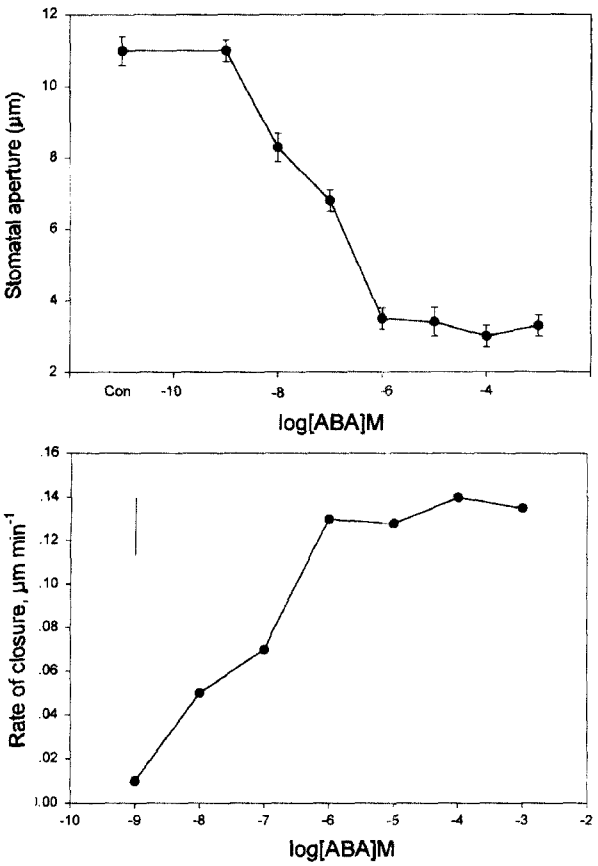

Figure 2. The effect of ABA on stomatal closure in isolated epidermis of *C. communis* L. Samples were incubated in 10 mM MES-KOH buffer (pH 6.2, including 50 mM KCl). Each point is the mean (±s.e.m.) of three replicate experiments, and 80 stomatal apertures were measured. The bar in the bottom figure indicates the least significant difference (L.S.D. = 0.023). In the bottom figure, stomatal apertures were simply converted to the rate of stomatal closure (μm min^{-1}), with the same results as in the top figure.

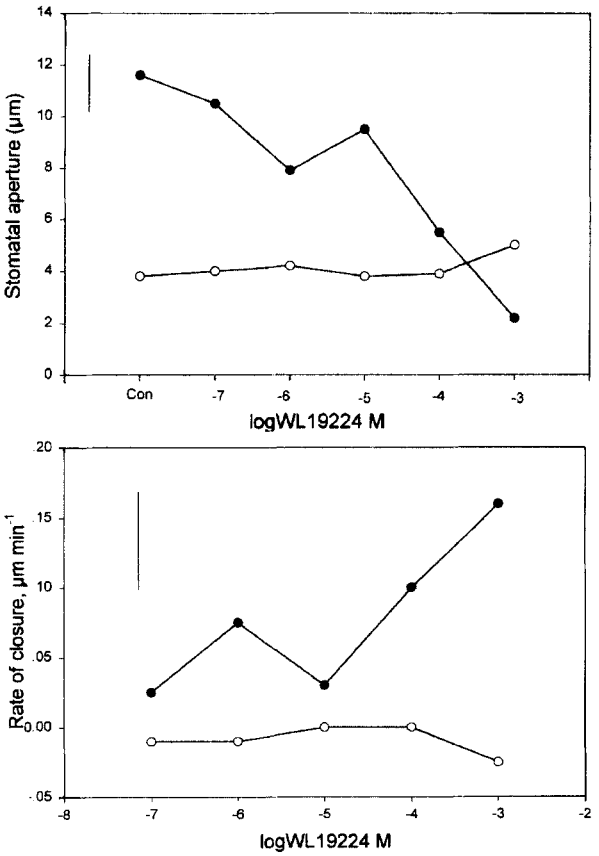

Figure 3. The response of stomata to WL19224 (closed circles) and WL19224 + ABA (open circles). Each point is the mean of three replicate experiments, and 80 stomatal apertures were measured. The bars in the bottom and top figures indicate the least significant differences, which are 2.13 and 0.065, respectively. In the bottom figure, stomatal apertures were simply converted to the rate of stomatal closure (μm min^{-1}), with the same results as in the top figure. Con = control. Control in closed circles indicates that WL19224 was not added; control in open circles indicates that 10^{-6} M ABA was singly added.

with 10^{-6} M ABA, is shown in Figure 3. This analogue produced a response similar to that of ABA. However, the magnitude of this dose response was slightly different. ABA was more effective at low concentrations and also at concentrations from 10^{-6} M to 10^{-4} M.

The combined treatment of ABA + WL19224 (from 10^{-7} M to 10^{-4} M) also induced stomatal closure, at a closing rate of about zero. This combination was as effective as that of ABA applied singly. In addition, when the stomatal apertures were enlarged as a result of the treatment of ABA + WL19224, the stomatal closing rate became negative compared with the controls. This indicates that concentrations of WL19224 from 10^{-7} M to 10^{-4} M did not inhibit or stimulate the effect of ABA.

At concentrations of 10^{-3} M for either WL19224 and ABA, stomata closed to 2.2 μm and about 3 μm, respectively. With a treatment of 10^{-6} M ABA only, stomata closed to about 3.5 μm. However, when a combination of 10^{-6} M ABA and 10^{-3} M WL19224 was applied to the epidermal strips floating on the incubation medium, the average stomatal aperture was 5 μm, with a least significant difference of 2.13. This indicates that the result of treating the strips with 10^{-3} M WL19224 was significantly different from the result in the combined treatment of ABA + WL19224. Therefore, WL19224 and ABA may possibly inhibit each other's effect at high concentrations.

The structural requirements for biological activity of ABA include carboxyl groups, tertiary hydroxy groups, and cis and ring double bonds. WL19224 is the analogue most similar in structure to ABA. This similarity could be the factor that induced the same stomatal closure observed with ABA. However, it is unknown which parts of the WL19224 structure are active during stomatal closure. Possible candidates are the two carboxyl groups, the methoxy (MeO), ring double bonds, or the entire structure. Because methoxy is the toxic functional group, it is the most likely candidate.

Figure 4 shows the effect on stomatal closure after treatment with either WL19377 alone, or when combined 10^{-6} ABA. This ABA analogue did not stimulate closure. In fact, at concentrations higher than 10^{-4} M, WL19377 had the reverse effect, causing approxi-

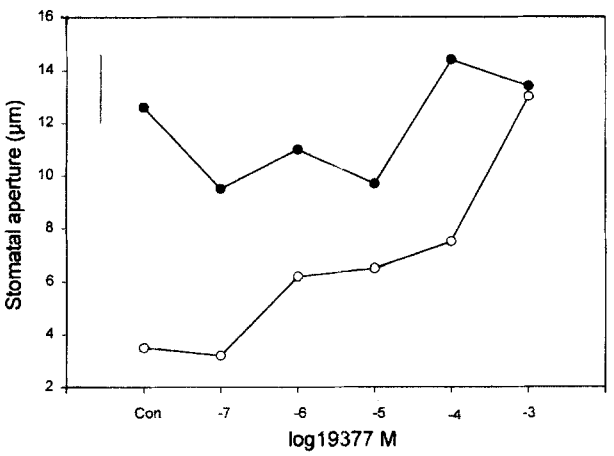

Figure 4. The response of stomata to WL19377 (closed circles) and WL19377 + ABA (open circles). Each point is the mean of three replicate experiments, and 80 stomatal apertures were measured. The bar in the figure indicates the least significant difference (2.13).

mately 10% stomatal opening compared with the control. In the combined treatment, ABA-induced stomatal closure was inhibited. This inhibitive effect of WL19377 on ABA was linear, according to concentration. Therefore, WL19377 could compete with ABA to bind the ABA receptor, thereby blocking the role of ABA in regulating stomatal activity. WL19377 also has a carboxyl group, double ring, and hydroxy group, all of which are necessary for controlling the activity of ABA.

In conclusion, the entire structure required for promoting biological activity of ABA and its analogues should be considered, rather than assessing only portions of that structure. For example, it has been demonstrated that although salicylic acid has a quite different structure compared with ABA, it is also very effective in stimulating stomatal closure (Lee, 1995).

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LITERATURE CITED

Cummins WR, Sondheimer E (1973) Activity of the asymmetric isomers of abscisic acid in a rapid bioassay. Planta 122: 307-310

Davies PJ (1987) Plant hormones and their role in plant growth and development. Martinus Nijhoff, Dordrecht, The Netherlands

Kriedemann PE, Loveys BR, Fuller GL, Leopold AC (1972) Abscisic acid and stomatal regulation. Plant Physiol 49: 842-847

Lee JS (1995) Effect of salicylic acid and its analogues on stomatal closing in *Commelina communis* L. J Korean Environ Sci Soc 4: 317-321

Lee JS, Bowling DJF (1992) Effects of the mesophyll on stomatal opening in *Commelina communis*. J Exp Bot 43: 951-957

MacRobbie EAC (1992) Calcium and ABA-induced stomatal closure. Phil Trans R Soc Lond B 338: 5-18

Milborrow BV (1966) The effects of synthetic dormin (abscisin) on the growth of the oat mesocotyl. Planta 70: 155-171

Mousseron-Canet M, Mani JC, Durand B, Nitsch J, Dornand J, Bonnafous JC (1970) Analogues de l'acide abscique (±) hormone de dormance. C R Acad Sci Paris 270: 1936-1939

Oritani T, Yamashita K (1970a) Studies on abscisic acid. Part I. Synthesis of keto-ionylideneacetic acids. Agr Biol Chem 34: 108-14

Oritani T, Yamashita K (1970b) Studies on abscisic acid. Part II. The oxidation products of metyl α- and β-cyclocitrylidene-acetates and methyl α-cyclogeranate. Agr Biol Chem 34: 198-203

Raschke K (1987) Action of abscisic acid on guard cells, *In* E Zeiger, GD Farquhar, IR Cowan, eds, Stomatal Function, Stanford University Press, Stanford, California, pp 253-279

Sondheimer E, Walton DC (1970) Structure-activity correlation with compounds related to abscisic acid. Plant Physiol 45: 244-248