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Action of Malformin A_1 on Gravitropic Curvature in Primary Roots of Maize (*Zea mays* L.)

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Malformins, a small family of cyclic pentapeptides, are active plant growth regulators isolated from the fungus *Aspergillus niger*. We purified malformin A_1 from the crude malformin A mixture, and studied its action in the gravitropic response of maize roots. Intact primary roots that had been pretreated vertically with malformin A_1 were placed in a humidified box in the horizontal position. Positive curvature (downward) was inhibited in the pretreated roots compared with the control. In addition, we measured the lateral transport of IAA in primary roots. Roots pretreated with malformin A_1 did not show asymmetric distribution of IAA between the upper and lower sides of the elongation zone. Malformin A_1 also stimulated ethylene production in maize root segments. Our results had suggested that malformin A_1 might inhibit the lateral transport of IAA across the roots from the upper to the lower side because of an increased level of ethylene. Therefore, we placed more IAA on the upper side at the initial phase of gravistimulation. These results were consistent with malformin A_1-pretreated roots showing inhibited positive gravitropic curvature.

Keywords: gravitropic curvature, IAA distribution, maize root, malformin A_1

The malformins, produced by the fungus *Aspergillus niger*, induce striking disfigurements during growth in higher plants. These highly active compounds comprise a small family of cyclic pentapeptides (Takahashi and Curtis, 1961). Malformin causes epinasty, swelling and reduced growth rates in shoots, curvatures and reduced growth rates in roots, and leaf abscission (Curtis, 1958a, 1958b). However, the physiological effects of malformin might be mediated by the activity of ethylene and phytochrome (Curtis, 1977).

Most of the earlier research was performed with crude malformin extracts. Although study of malformins in plants did not continue after the late 1970s, work has been recent and ongoing in the animal and medical sciences. There, malformin A_1 has inhibited the binding of interleukin-1 beta to endothelial cells and monocytes in humans (Herbert et al., 1994). In addition, malformin A_1 and nodularins from cyanobacteria have inhibited the activity of phosphatase (Bagu et al., 1997). Recently, the structures of 9 kinds of malformin have been studied extensively (Kim et al., 1993a, 1993b). Among them, malformin A_1 is the most active compound in its physiological effects. Its structure is cyclo-L-isoleucyl-D-cysteinyl-L-valyl-D-cysteinyl-D-leucyl (Fig. 1). In most studies, malformin A has been applied as a mixture of 84% A_1 and 11% A_2. Therefore, the research presented here focused, instead, on the effect of purified malformin A_1, rather than malformin A mixtures or crude malformin extracts.

Gravitropism is a differential growth response that is induced either by a gravitational stimulus or by mass acceleration (e.g., centrifugation). Roots, particularly those that are primary, exhibit a positive (downward) gravitropic response, whereas secondary and tertiary roots often grow almost horizontally. Roots follow a sequence of events in their response to gravitational stimuli: (1) the gravitational vector is perceived by either a statolith or an unknown sensor system in the root cap or tip (perception); (2) ion- and/or hormone-gating channels are depolarized (transduction); and (3) the asymmetrical elongation results in differential growth across the axis of the root (response).

The gravitropic response of the roots occurs in the elongation zone, 2 to 6 mm behind the root cap. Therefore, some signal must connect the root cap to this zone. The Cholodny-Went hypothesis has proposed that gravicurvature of roots results from growth inhibitors accumulating along the lower side of a horizontally oriented root. Evidence suggests that the growth inhibitor might be either auxin (Steen and Hild, 1980; Jackson and Barlow, 1981; Mulkey and Evans, 1981, 1982; Kim and Mulkey, 1997b) or ABA (Pilet and Chanson, 1981). It is widely accepted that

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auxin plays the central role in the asymmetric elongation rates observed during gravicurvature (Lee et al., 1990; Kim and Mulkey, 1997b; Rosen et al., 1999).

Ethylene, another important mediator of root gravitropism, is a gaseous plant hormone that regulates some physiological responses (Abeles et al., 1992; Kende, 1993). It stimulates the ripening of fruit (Theologis, 1992) and inhibits stem and root elongation. Ethylene production is prompted by a plant hormone such as auxin, as well as by physical injury (Lee et al., 1999). Altering its production may, in turn, control the elongation of roots in maize (Kim and Mulkey, 1997a, 1997b).

In the current study, we tested the effect of two concentrations of purified malformin A_1 on the gravitropic response in the primary roots of maize (*Zea mays*). We also attempted to determine whether the changes in gravitropic curvature in the malformin A_1-pretreated roots were due to ethylene-induced auxin redistribution in the elongation zone. To do so, we measured ethylene production and the ratio of radioactive IAA contents between the upper and lower sides of the roots after gravistimulation.

MATERIAL AND METHODS

Plant Material

Maize seeds (*Z. mays* L., Golden cross Bantam 70) were soaked overnight at 25°C and germinated in the vertical position between wet paper towels on opaque plastic trays. The trays were kept in a dark growth chamber at 25°C. We used two-day-old seedlings with primary roots that were about 15 to 20 mm long.

Purification of Malformin A_1

A solution of crude malformin A in DMSO was subjected to HPLC analysis on an ODS column (Capcell Pak C-18, 10 × 250 mm). We isolated, in pure form, four peaks that we had observed under both UV (252 nm) and an RI detector with an eluent of methanol-water-TFA (70:30:0.5). The purified form of malformin A_1 is shown in Figure 1.

Measurement of Gravitropic Curvature

Roots were pretreated vertically for 1 h with either 1 µM or 0.1 µM malformin A_1, after which the seedlings were transferred to a humidified chamber (>95% RH) and placed in the horizontal position.

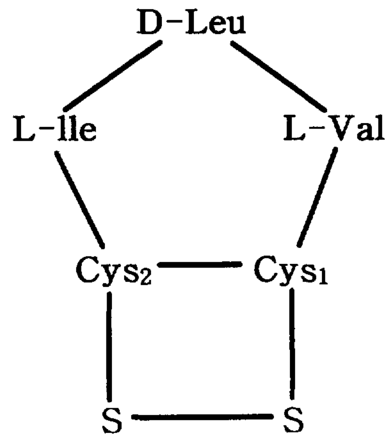

Figure 1. Structure of malformin A_1. Molecular weight is 529. The amino acid composition ratio is Val(1):Leu(1):Ile(1):Cys(2).

We measured the pattern of gravitropic curvature for 7 h, using a time-lapse video cassette recorder (Samsung, STLU-36D, Korea) equipped with a CCD camera (Samsung Aerospace, SAC-410NDX, Korea). The time-lapse series pictures were analyzed with an Image Tool Program.

Analysis of IAA Distribution

To measure the distribution of IAA across the elongation zone, ^{3}H-IAA was incorporated into agar blocks (1.0% agar, 1 mm^3 cube). These radioactive donor blocks were placed on either the top or bottom side of the root. A plain agar receiver block of the same dimensions was placed on the opposite side of the root, directly across from the donor block. The roots were pretreated with malformin A_1 for 1 h before being transferred to a humidified chamber. After a period to allow for auxin transport, the receiver blocks from the bottoms or tops were placed in scintillation vials, and the ratio of radioactivity in the receiver blocks (bottom/top) was then calculated to determine the extent of IAA distribution.

Measurement of Ethylene Production

Root segments, including the root tip (10 mm), were incubated at 27°C in the dark in 25-mL vials sealed with silicon stoppers, using a 0.5-mL potassium phosphate buffer (0.05 M; pH 6.8). A 1-mL gas sample was withdrawn and analyzed with a gas chromatograph (DS 6200, Donam Instrument Inc., Korea) that was equipped with an aluminum column and a flame-ionization detector at 180°C.

RESULTS AND DISCUSSION

Purification of Malformin A_1

HPLC analysis of crude malformin A identified four peaks for malformin A_1, A_2, A_3, and A_4 (Fig. 2). This was consistent with the results of Kim et al. (1993). Four peaks also were observed under both UV (252 nm) and RI detectors. One major peak and one of the minor peaks (in the ratio of 84:11 calculated-peak area) could be attributed to A_1 and A_2.

Effect of Malformin A_1 on Gravitropic Response

Roots pretreated vertically with malformin A_1 for 1 h showed inhibition of positive (downward) curvature within 2 h (Fig. 3). In particular, roots pretreated with 1 µM malformin A_1 showed a negative (upward) curvature during this phase. These roots then showed more positive curvature, to 20-35°, over the next 3 h. After 7 h, the curvature was inhibited by only 20%, compared with the control. Roots pretreated with 0.1 µM malformin A_1 also showed less positive curvature than did the control roots within the first 2 h; this inhibition was maintained over the next 3 h as well. After 7 h, curvature was inhibited by only about 20%, compared with the control. The period of inhibition was extended for roots pretreated with 0.1 µM malformin A_1, which suggests that that level may be the more effective concentration for inhibiting gravitropic curvature.

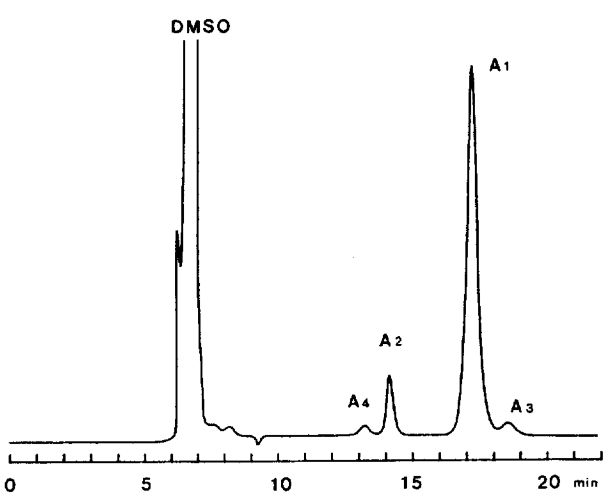

Figure 2. Purification of malformin A_1 from a crude malformin A mixture by HPLC.
A_1: retention time; 17.2 min peak area; 84%
A_2: retention time; 14.1 min peak area; 11%
A_3: retention time; 18.5 min peak area; 4%
A_4: retention time; 13.3 min peak area; 1%

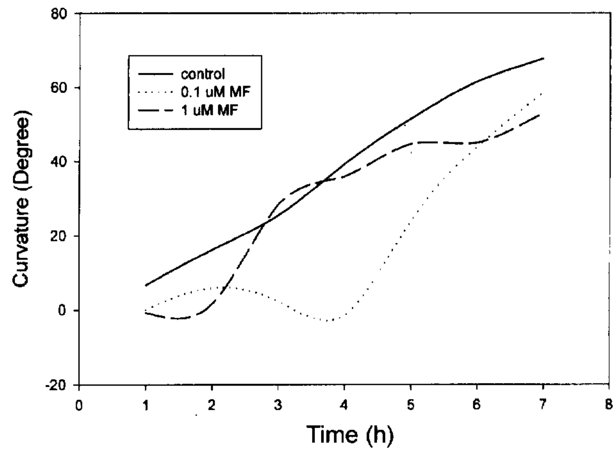

Figure 3. Effect of malformin A_1 on the gravicurvature in intact primary roots of maize. Roots were pretreated for 1 h in the vertical position with 1 µM malformin A_1 (dashed line) and 0.1 µM malformin A_1 (dotted line). Control roots (solid line) were pretreated vertically with buffer for 1 h. All pretreated roots were transferred to the humidified box, in a horizontal position, for 8 h (n>45).

Our results contradict a report that the 1-µM concentration of malformin was most effective in stimulating stem growth under white light (Curtis, 1977). Therefore, purified malformin A_1 may exert a physiological effect at a lower concentration than was previous believed. In addition, higher concentrations may, in fact, be toxic to plants. Further experiments are needed to confirm this possibility.

We believe that roots pretreated with either concentration of malformin A_1 initially showed inhibition of positive curvature, while exhibiting more oscillation than did the control. That is, the malformin A_1-pretreated roots seemed to have lost their ability to detect gravity. Our conclusions differ from those in earlier studies. For example, Izhar et al. (1969) reported that the positive (downward) curvature in maize roots was increased, but ethylene production was not stimulated, by applications of malformin. This difference might be explained by the fact that they had used a crude culture filtrate of malformin from *A. niger*, which may have been a mixture of malformin A and B. Therefore, further study of the physiological effects of purified malformin A_1 in plant tissues should revisit these previous results.

Effect of Malformin A_1 on the Lateral Transport of ^{3}H-IAA

Both concentrations of malformin A_1 inhibited positive curvature in primary roots of maize (Fig. 3). Two explanations are possible. First, according to the Cholodny-Went hypothesis, gravitropism in roots results from auxin accumulating on the lower side of the elongation zone. Roots are very sensitive to auxin (Thimann, 1936), so their growth would normally be inhibited on that lower side, causing a positive curvature. Because pretreatment with malformin A_1 may have reduced auxin sensitivity in gravistimulated roots, that initial accumulation of auxin would not have been sufficient to inhibit growth on the lower side, thereby preventing positive curvature. Further studies are needed to confirm this possibility.

Another explanation is that pretreatment with malformin A_1 may have affected the lateral auxin transport (LAT) system in the gravistimulated roots. Hild and Hertel (1972) showed that roots demonstrating negative curvature (upward) during the initial phase may have had such a greater sensitivity to gravity that the graviperception signal caused overstimulation of the LAT system. This overstimulation may have prompted transient auxin transport toward the upper side of the elongation zone, which resulted in growth inhibition and negative curvature. The subsequently positive curvature occurred after the roots had adapted their LAT system.

To confirm the results of negative (or inhibited) curvature in malformin A_1-pretreated roots, LAT was measured in the elongation zone every 30 min (Fig. 4). The ratio after 1 h of transport across the control roots was about 1.04; for 0.1 µM- and 1 µM-malformin A_1-pretreated roots, ratios were about 0.92 and 1.00, respectively. We can infer from this that more IAA accumulated within the first hour on the upper side of the malformin-pretreated roots, resulting in the inhibited positive curvature shown in Figure 3. In contrast, the control roots showed asymmetrical distributions of IAA within the first hour.

Within 2.5 h, the control roots had differential, asymmetrical distribution of IAA in the elongation zone, with a ratio of 1.18 (Fig. 4). That is, LAT caused more radioactive IAA to accumulate on the lower side of the control root. This accumulation may have inhibited elongation on that side, so that the roots would have shown positive (downward) curvature. In contrast, roots pretreated with either concentration of malformin A_1 had lower ratios, approximately 1.03. Malformin A_1 probably inhibited the asymmetrical distribution of IAA, resulting in no significant differential growth across the axis in the horizontally oriented root. Therefore, positive gravitropic curvature was inhibited within 2 h of gravistimulation in the malformin-pretreated roots, compared with the control roots (Fig. 3).

Although the ratios for the 0.1 µM and 1 µM malformin A_1-pretreated roots did not differ significantly after 1.5 h, the kinetics of the gravitropic curvature were different after 2 h (Fig. 3). It is possible that the extent to which root tips in the first hour lost most or all of their ability to detect gravity depended on the concentration of malformin A_1. This possibility could be tested by investigating the redistribution of calcium in root tips within 1 h of pretreatment with various concentrations of malformin A_1.

Effect of Malformin A_1 on Ethylene Production

The inhibition of root elongation can be reversed by treatment with ethylene antagonists (Kim and Mulkey, 1997b). LAT also can be inhibited by ethylene (Abeles et al., 1992). Lee et al. (1990) have suggested that ethylene can modify positive curvature in the primary roots of maize by affecting their gravity-induced LAT. This is, perhaps, accomplished by interfering with the root's adaptation of its auxin transport system to the gravistimulus. Our research also showed that pretreatment with malformin A_1 caused inhibition of LAT (Fig. 4).

To explain the relationship between ethylene and LAT when roots were pretreated with malformin A_1, we measured IAA-induced ethylene production (Fig.

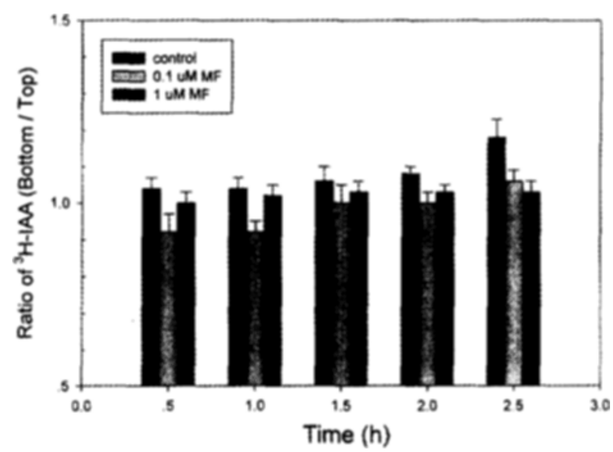

Figure 4. Time course of the development of IAA asymmetrical distribution between the upper and lower sides of the elongation zone in horizontal primary roots. Roots were pretreated with malformin A_1 (0.1 µM or 1 µM) for 1 h prior to being placed in the horizontal position. The ratios that are >1.0 indicate that IAA distribution is asymmetrical (IAA mainly distributed on the lower side in the elongation zone of the horizontally-oriented roots) (n>40).

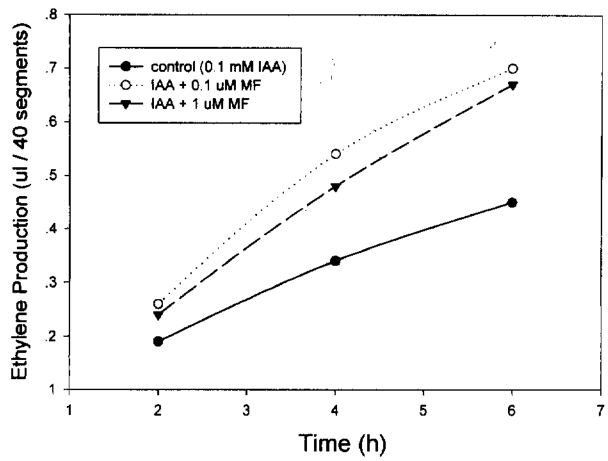

Figure 5. Kinetics of 0.1 mM IAA-induced ethylene production in the presence of malformin A_1 (0.1 μM or 1 μM) in the primary roots of maize (n>60).

5). Ethylene levels increased within 2 h, with production being about 155% of the control in the presence of either concentration of malformin A_1. Therefore, we might conclude that malformin A_1 increased ethylene production in roots, thereby altering or inhibiting LAT. This inhibition would have delayed or deterred positive (downward) curvature in the malformin-pretreated roots.

Further study is required for describing the effect of malformin A_1 on ethylene production in maize roots. Those tests would focus on the action of malformin A_1 in ethylene biosynthesis, including monitoring the activity of ACC oxidase as well as determining the ACC content in the primary roots. Additional experiments could also investigate the effects of ethylene levels on gravitropic curvature.

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