

Promotion of Growth and Hydrogen Ion Efflux by Auxin in Roots of Maize Pretreated with Ethylene Biosynthesis Inhibitors¹

Received for publication November 16, 1981 and in revised form February 16, 1982

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ABSTRACT

Low concentrations of auxin (e.g. 10^{-10} M) do not promote the growth of intact seedling roots of maize (*Zea mays* L. Bear Hybrid WF 9 × 38). Higher concentrations are inhibitory. When the roots are pretreated with the ethylene biosynthesis inhibitors, cobalt and aminoethoxyvinylglycine, auxin (10^{-10} to 10^{-8} M) strongly promotes their growth. The promotion of growth by auxin in pretreated roots is preceded by enhanced hydrogen ion secretion from the roots. The data indicate that hormone-enhanced hydrogen ion secretion may play a role in the rapid promotion of root growth by auxin. The ability of auxin to promote the growth of intact roots is discussed in relation to the Cholodny/Went hypothesis of hormonal control of root geotropism.

auxin in concentrations from 10^{-10} to 10^{-8} M promotes elongation in maize roots which have been pretreated with ethylene biosynthesis inhibitors (15). Using this method, we have looked for auxin-induced H^+ efflux and compared the concentration dependence of the action of auxin on growth and H^+ movement in roots.

MATERIALS AND METHODS

Plant Material. Grains of maize (*Zea mays* L., Bear Hybrid WF 9 × 38, Customaize, Momence, IL) were soaked in tapwater for 8 h and placed between wet (demineralized H_2O) paper towels on plastic trays (24 × 32 × 1.5 cm, length, width, height). The trays were placed in a vertical position with the grains aligned along the vertical axis. The seeds germinated at room temperature (20–24°C) under fluorescent room lighting (intensity approximately $175 \mu E/m^2 \cdot s$). The seedlings were used 3.5 d after planting.

Measurement of Elongation. Growth was measured as increase in length of the root using intact seedlings mounted in a root auxanometer (6). The auxanometer was modified so that the output was directed both to a recorder and to an Imsai PCS-42 microcomputer. The auxanometer is based upon a rotary variable differential transformer which produces a linear change in voltage proportional to displacement.

Computer measurements of growth were made by analog to digital conversion of the transducer output voltage. Resolution was $\pm 2 \mu m$ with repeatability of $\pm 4 \mu m$. Mean value measurements of less than 3% relative error were stored every 20 s for further analysis. Measurements were made at the rate of 500/s. Growth rate was calculated based upon the change in relative length over time periods of 20 and 60 s.

Measurement of H^+ Efflux. Measurement of H^+ efflux was done as described by Evans *et al.* (8). Briefly, 60 1-cm apical sections of primary roots are placed in 3.5 ml 1 mM K-phosphate buffer (initial pH 6.3). A semi-micro combination pH electrode is inserted into the medium. Output from the electrode is recorded on a recorder adjusted to give full scale displacement for 1 pH unit. The medium is continuously oxygenated (pure O_2 , 0.43 L/min), and stirred by a spin bar separated from the sections by a plastic screen.

Measurement of Ethylene in Root Sections Pretreated with Co/AVG.² To measure ethylene in root sections pretreated with Co/AVG, 80 1-cm apical root sections were cut and placed immediately into a 3 ml vial containing 1 mM K-phosphate buffer (pH 6.4) with or without Co/AVG. The vial was flushed with O_2 , sealed with a septum, and held at 24°C. After 3 h, gas samples were removed from the vial and analyzed by GC using a Hewlett Packard Model 5750 gas chromatograph with a 1.83 m × 0.318 cm alumina-packed column. The column was used in the isothermal mode at 100°C, with the injection port and flame ionization temperatures both 110°C. Nitrogen was used as the carrier gas.

According to the acid growth hypothesis, the rapid promotion of cell elongation by auxin is mediated by hormone-induced acid efflux into the cell wall (10, 17, 18). There is considerable evidence in favor of this hypothesis, especially as applied to stem tissue (17). There is less evidence that auxin action on roots is mediated by H^+ movement. We have shown that growth-inhibiting concentrations of auxin cause H^+ uptake by roots (8), but evidence that growth-promoting concentrations of auxin can stimulate acid efflux in roots is lacking. Experiments to test such a relationship are difficult to perform, inasmuch as applied auxin does not consistently promote root growth. This is especially true of intact roots (1) where applied auxin either inhibits growth or has no effect, depending on concentration (15). In auxin-depleted root sections, applied auxin sometimes promotes growth, but the effect is often short-lived or restricted to a very narrow concentration range (5, 16).

In spite of these difficulties, there is evidence that wall pH plays a role in the control of root growth. Acid pH stimulates the growth of roots (4), whereas neutral buffers are inhibitory (12). Also, there is rapid H^+ efflux from the elongation zone of intact maize roots (13), and concentrations of auxin which inhibit root growth, inhibit or reverse this acid efflux (8, 13).

Recently, Moloney *et al.* (12) showed that antiauxins promote growth and H^+ efflux in apical segments of maize roots. They interpret the data to indicate that antiauxins bring the effective endogenous level of auxin from a supraoptimal to a near optimal value and that this leads to enhanced H^+ efflux and growth in a cause-effect relationship.

Studies of auxin promotion of root growth are complicated by the fact that auxin rapidly induces the biosynthesis of ethylene, an inhibitor of root elongation (3, 19). We recently reported that

¹ Supported by National Science Foundation Grant PCM 8103298.

² Abbreviations: AVG, aminoethoxyvinylglycine; Co/AVG, a solution of 10^{-4} M cobalt nitrate and 10^{-6} M aminoethoxyvinylglycine.

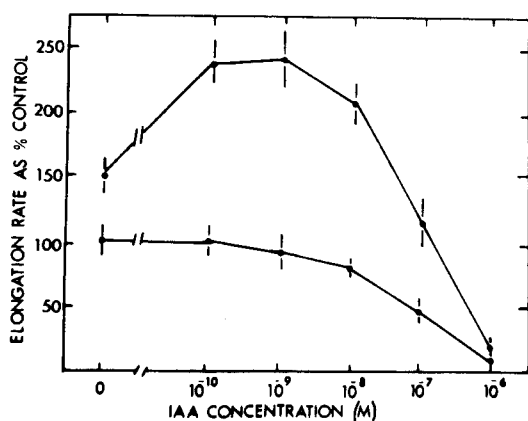


FIG. 1. Concentration dependence of IAA action on the elongation rate of intact roots of maize pretreated with Co/AVG. Upper line, roots pretreated with 10^{-4} M cobalt nitrate plus 10^{-6} M AVG for 1 h and then transferred to the indicated concentration of IAA (plus Co/AVG). Growth rate was measured 2 h after transfer to the IAA-containing solutions. Lower line, control roots held in buffer for 1 h and then treated with IAA. Growth rate measured 2 h after transfer to IAA.

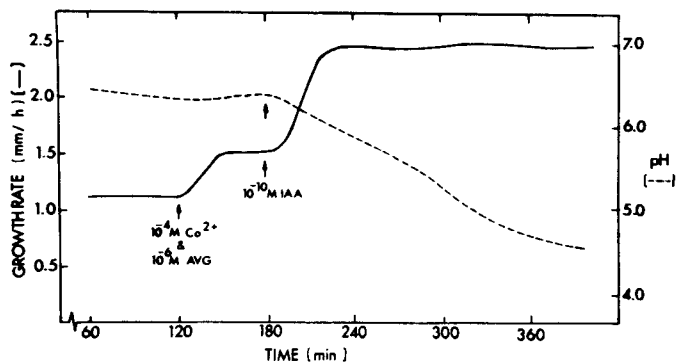


FIG. 2. Promotion of growth and H⁺ secretion by 10^{-10} M IAA in maize roots pretreated with Co/AVG. (---), 80 1-cm apical root sections cut and held in 1 mM K-phosphate buffer (initial pH 6.3) plus 10^{-4} M cobalt nitrate and 10^{-6} M AVG for 3 h before addition of auxin (arrow). (—), growth rate of a single intact root. The root was pretreated with Co/AVG beginning at the first arrow, and IAA (10^{-10} M) was added to the pretreatment solution at the second arrow.

Chemicals. IAA was purchased from Sigma. AVG was obtained through the courtesy of Dr. G. Lee Benson, Maag Agrochemicals, HLR Sciences, Inc., Vero Beach, FL, and from samples kindly provided by Dr. Richard Gladon, Department of Horticulture, Iowa State University. In all cases, AVG was used at 10^{-6} M. This is below the concentration (10^{-4} M) at which AVG begins to inhibit protein synthesis (11), and, in fact, we find that 10^{-6} M AVG promotes root growth slightly (15).

RESULTS

The effect of IAA on the growth of intact roots of maize with and without Co/AVG pretreatment is shown in Figure 1 (also see Ref. 15). In nonpretreated roots, 10^{-10} M IAA has no effect on elongation, whereas higher concentrations are inhibitory. In pretreated roots, ethylene levels are reduced by about 75% (15) and, under these conditions, IAA from 10^{-10} to 10^{-8} M strongly promotes elongation.

When ethylene accumulation was measured using apical root

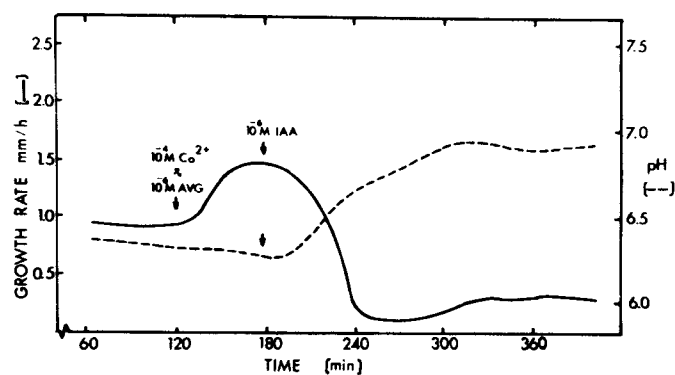


FIG. 3. Inhibition of growth and induction of H⁺ uptake by 10^{-6} M IAA in maize roots pretreated with cobalt and AVG. Experimental details were the same as for the experiments of Fig. 2 except that 10^{-6} M IAA was used instead of 10^{-10} M IAA.

Table 1. Concentration Dependence of IAA Action on Growth and H⁺ Movement in Roots Pretreated with Cobalt and AVG

Log of IAA Concentration	Promotion (+) or Inhibition (-) of Growth Rate Relative to Control ^a	Rate of Change in External pH
M	%	pH units/h
-5	-94	+0.56
-6	-81	+0.32
-7	+22 ^b	+0.3/-0.43 ^c
-8	+108	-0.42
-9	+137	-0.58
-10	+137	-0.58
-infin	+53	

^a The control rate of elongation is taken as that of roots receiving neither IAA nor Co/AVG. The calculation is based on the rate of elongation 2 h after addition of IAA compared with the steady rate of elongation prior to addition of either IAA or Co/AVG.

^b The initial effect of addition of 10^{-7} M IAA to Co/AVG pretreated roots is to cause an inhibition of growth. This inhibition reaches a maximum after about 40 min. The growth rate then recovers during the following 45 min reaching a steady rate about 22% higher than the rate in control roots receiving neither Co/AVG nor IAA.

^c The pH of the medium surrounding pretreated root sections to which 10^{-7} M IAA is added, increases during the first h and then decreases. The first value in the pH shift column indicates the rate of pH rise during the first phase. The second value indicates the rate of pH drop during the second phase. This biphasic pH response is paralleled by a biphasic growth response (see footnote b).

sections held in sealed vials as described under "Materials and Methods," large differences were found in ethylene released from control roots versus roots treated with Co/AVG. After 3 h, the ethylene content (98 ± 12 nl/L) of vials containing control sections was nearly 3 times that of vials containing Co/AVG-treated sections (34 ± 5 nl/L).

The effect of a growth-promoting concentration of auxin (10^{-10} M) on H⁺ efflux from Co/AVG-pretreated apical root sections is shown in Figure 2. IAA at 10^{-10} M causes strong acidification of the medium containing the sections, with a latent period of 4 to 9 min. For purposes of comparison, the effect of 10^{-10} M IAA on the growth of pretreated roots is also shown in Figure 2. The hormone strongly promotes elongation, with a latent period which varies from 8 to 12 min.

Figure 3 shows the effect of 10^{-6} M IAA on H⁺ movement and growth in Co/AVG-pretreated roots. IAA at 10^{-6} M induces a

strong apparent uptake of H^+ , causing the pH of the medium to increase. This increase in pH of the medium is paralleled by a decrease in the growth rate of intact roots treated with the same concentration of IAA (Fig. 3). Table I summarizes the effect of a range of auxin concentrations on growth and H^+ influx or efflux in roots pretreated with Co/AVG. There is a close correlation between the effect of the hormone on growth and its effect on H^+ movement. Concentrations of IAA which stimulate growth also stimulate H^+ efflux, while concentrations which inhibit growth cause an apparent uptake of H^+ by the root.

DISCUSSION

It is commonly suggested that the difficulty in obtaining promotion of intact root elongation by auxin arises because the internal level of auxin is optimal or supraoptimal so that addition of auxin cannot further stimulate growth. An alternative explanation is that the applied hormone may rapidly induce the synthesis of ethylene, an inhibitor of root elongation (15). The data presented here and in Mulkey *et al.* (15) are consistent with the latter interpretation. When ethylene biosynthesis is suppressed, IAA strongly promotes elongation in intact roots over at least a one hundred fold concentration range. This indicates that the auxin level *per se* is not supraoptimal in the roots of this variety of maize but may be at a level where additional auxin enhances ethylene production, leading to suppression or masking of a potential growth promoting influence of the applied IAA.

We find that auxin action on the growth of Co/AVG pretreated roots is closely paralleled by effects on H^+ efflux or uptake. In pretreated roots, low concentrations of auxin promote both growth and H^+ efflux. This contrasts with the findings for non-pretreated roots in which low concentrations of auxin (e.g. 10^{-10} M) affect neither growth nor H^+ secretion. High concentrations of auxin (e.g. 10^{-6} M) inhibit growth and cause H^+ uptake in either pretreated or control roots (Fig. 3; Ref. 8).

These observations, together with evidence that acid buffers promote (4) and neutral buffers inhibit root growth (12), suggest that the rapid action of auxin on roots may be mediated by H^+ secretion. This plus the similarity in the latent period of auxin promotion of root (8–12 min) and stem (6–15 min) growth (7), indicates that the hormone may be acting in a similar manner in roots and stems, as proposed by Thimann (21).

The finding that, under special conditions, auxin can consistently promote the growth of intact roots, does not necessarily conflict with the Cholodny/Went hypothesis of auxin mediation of root geotropism. Roots are not normally exposed to excess cobalt or to AVG, so it may be that a localized increase in auxin concentration within the root normally leads to growth inhibition as assumed by the Cholodny/Went hypothesis. However, the inhibition may arise more from increased ethylene production than from establishment of a supraoptimal concentration of auxin *per se*. According to this idea, root geotropism would be triggered by movement of auxin to the lower half of the root. The increased concentration of auxin would lead to enhanced ethylene production, resulting in a reduction in the growth rate of the lower cells. This model was proposed earlier by Chadwick and Burg (3) in a study of ethylene and auxin interaction in the growth and geotropism of pea roots. They noted that auxin rapidly induces the formation of ethylene in pea roots and that CO_2 , an ethylene antagonist, impedes their geotropic response. This model was later refuted by Andreae *et al.* (2), primarily on the basis of kinetic studies of auxin and ethylene inhibition of root growth. Our results are consistent with the model proposed by Chadwick and Burg. As additional support for their model, we have noted (unpublished data) that pretreatment of roots with 10^{-4} M AVG eliminates their geotropic sensitivity.

Because of the recent evidence that asymmetric acid efflux plays a role in initiating geotropic curvature in roots (13), one might speculate that redistribution of auxin, combined with auxin-induced establishment of unequal ethylene levels across the root,

mediates the development of the differential acid efflux pattern. This would be consistent with the observations that: (a) low levels of auxin stimulate H^+ efflux from roots treated to suppress ethylene biosynthesis; (b) higher levels of auxin inhibit or reverse H^+ efflux from roots; (c) asymmetric acid efflux (most intense on the upper surface) precedes geotropic curvature in maize roots (13), and (d) treatment of roots with acidic solutions accelerates their growth and greatly delays their geotropic response (20). Asymmetric acid efflux may also mediate shoot geotropism since: (a) auxin stimulates acid efflux from stems (17), (b) asymmetric acid efflux (most intense on the lower surface) precedes geotropic curvature in shoots (14), and (c) neutral buffers prevent geotropic curvature in sunflower (*Helianthus*) hypocotyl segments (23). It is not clear how ethylene production might be involved in shoot geotropism, if at all. Wheeler and Salisbury (22) found that inhibitors of ethylene biosynthesis delay the geotropic response in stems of *Xanthium strumarium* L., indicating that ethylene production plays some role in shoot geotropism, at least in this species.

Our results warrant further study of the involvement of ethylene in root and shoot geotropism. In particular, it will be helpful to obtain data on the effects of ethylene and ethylene biosynthesis inhibitors on both the development of asymmetric H^+ efflux patterns and the kinetics of curvature in geo-stimulated roots and shoots.

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