Auxin Inhibition of Acid- and Fusicoccin-induced Elongation in Lentil Roots

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Abstract. Both acid pH (4.0) and fusicoccin (FC) strongly stimulate root elongation in intact lentil (Lens culinaris Med.) seedlings. FC-induced elongation is apparently mediated by FC-enhanced H⁺ secretion since the toxin induces massive secretion of H⁺ in these roots after a latent period of less than 5 min. Auxin (indole-3-acetic acid) strongly inhibits elongation in control roots as well as acid-induced and FC-induced root elongation. Treatment of apical root segments with auxin causes only a slight apparent uptake of H⁺ and has no inhibitory effect on FC-induced H⁺ secretion, whether the hormone is given before or after the toxin. Auxin induces ethylene production in excised roots of lentil but the latent period is at least 30 min while inhibition of root elongation by IAA is maximal within 30 min. It is concluded that the inhibitory action of auxin on acid- and fusicoccin-induced root elongation is a direct effect, independent of auxin-induced ethylene production or auxin-mediated modification of cell-wall pH.

Key words: Acid growth – Auxin – Ethylene – Fusicoccin – Growth inhibition – Lens – Root growth.

Introduction

It is well established that auxin induces hydrogen-ion secretion in a number of plant tissues and that cell elongation in these tissues is strongly promoted by acid pH (Marré et al., 1972, 1975; Cleland, 1973; Rayle, 1973; Yamagata and Masuda, 1975). There is recent evidence that acidification in response to auxin accompanies or precedes growth promotion, indicating that enhancement of H⁺ secretion by auxin may mediate the action of the hormone in the promotion of cell elongation (Jacobs and Ray, 1976; Cleland, 1976).

The apparent correlation between auxin action and H⁺ secretion in stems and coleoptiles raises the question of the role played by H⁺ secretion in the growth of tissues that respond to auxin differently from stem tissues. For example, auxin is known to inhibit strongly the elongation of intact roots over a wide concentration range (Thimann, 1936; List, 1969; Evans, 1976b). Acid pH, on the other hand, has been shown to stimulate elongation in roots strongly (Edwards and Scott, 1974, 1976; Batra et al., 1975; Lado et al., 1976; Evans, 1976a) just as it does in stems. Pilet (1976) has recently demonstrated that auxin inhibits elongation in corn roots while the fungal toxin, fusicoccin (FC), strongly stimulates both elongation and H⁺ secretion in the same material. We have found (Evans, 1976a) that the inhibitory effect of IAA on root elongation dominates the promotive effect of acid pH, i.e. that auxin inhibits acid-induced elongation in intact roots. Auxin inhibition of acid-induced elongation of corn root segments has also been reported by Batra et al. (1975). The purpose of the present study was to determine the involvement, if any, of H⁺ movement in the inhibition of root elongation by auxin.

Materials and Methods

Most experiments were performed with roots of 3-day-old lentil (Lens culinaris Med.) seedlings. Seedlings were obtained by soaking lentil seeds (obtained at a local market) in tap water for 2-4 h and planting them in plastic trays of moistened vermiculite in the laboratory at room temperature with day-time lighting from General Electric cool-white fluorescent lamps (about 1400 erg cm⁻² s⁻¹). For purposes of comparison of root and coleoptile responses to acid one set of experiments was done using segments from coleoptiles of 3-day-old dark-grown corn (Zea mays L. Bear Hybrid WF 9 × 38) seedlings. The corn seedlings were obtained and segment elongation measured as described by Evans and Hockanson (1969).
Root-growth experiments were done using a single, intact seedling mounted in a root auxanometer as described earlier (Evans, 1976a). The root auxanometer utilizes an angular position-sensor transducer to monitor root elongation continuously while the root is submerged in an appropriate oxygenated medium. Unless otherwise noted the growth medium was half-strength Meyer's solution (Meyer et al., 1955) adjusted to pH 6.5 with NaOH.

Apparent H⁺ secretion or uptake was measured by monitoring the change in pH of 7 ml of medium (either water or Meyer's solution) containing 30 1-cm apical sections of 3-day-old roots. The pH electrode employed was a Fisher (Pittsburgh, Pa., USA) Microprobe Combination pH Electrode with a 6.25-mm tip diameter. The output of the electrode was recorded using a Sargent-Welch (Detroit, Mich., USA) Model SRLG recorder with full scale deflection adjusted to 0.5–1.0 pH units. In measurements of auxin effects on H⁺ uptake, a small volume of concentrated auxin was added to the medium to obtain a final concentration of 1 μM after mixing. Since the addition of auxin caused the pH to drop slightly the pH was immediately readjusted to the starting value using dilute NaOH.

Auxin-induced ethylene production in lentil roots was measured by gas chromatography. Ninety 1-cm apical sections were incubated in 1 μM IAA for 25 min and transferred without rinsing to 4.5-ml vials stoppered with serum caps and lined with wet filter paper. One-ml samples were taken at specified intervals and the vials were flushed with air after withdrawal of each sample. Ethylene in the sample was determined using a Hewlett-Packard (Cleveland, O., USA) Model 5750 gas chromatograph with flame detection and a 60 cm × 0.625 cm column of 30–60 mesh Al₂O₃ at 100°C. Under these conditions an ethylene concentration of 10 nl/1 was readily detectable in a 1-ml sample.

For experiments involving ethylene treatment of intact roots mounted in the auxanometer, aqueous solutions of Ethrel (2-chloroethylphosphonic acid; Amchem Products, Ambler, Pa., USA) were used.

Results

Stimulation of Root Elongation by Acid pH or FC

Figure 1 shows the response of the intact lentil root to acid pH and to 10⁻³ M FC. There is a strong increase in growth rate in response to either treatment (150% in response to pH 4.0 and 350% in response to FC), and in both cases the latent period is short (about 2 min in response to acid and about 6 min in response to FC). Figure 2 shows that FC also rapidly induces H⁺ secretion in lentil roots. The acidification in response to FC begins within 5 min, while the maximum rate of acidification is established within 20 min. In water the equilibrium pH obtained in the presence of FC is about 4.7 while in the presence of 2 mM KCl the final pH is about 4.2 (data not shown).

Inhibition of Acid- and FC-induced Elongation by Auxin

Figure 3 shows that both acid-induced (Fig. 3B) and FC-induced (Fig. 3A) root elongation are strongly inhibited by 1 μM IAA. The latent period of the inhibitory effect is about 12 min, with maximum inhibition after 20–40 min. These kinetics are similar to those for auxin inhibition of root elongation in control seedlings (Fig. 3C). As shown in Figure 3B, removal of auxin does not release the roots from auxin inhibition of acid growth, at least not during the subsequent hour. Acid (pH 4.0) added after thoroughly rinsing (5 washes) the auxin solution from the root chamber has little promotive effect on elongation. A similar prolonged period of inhibition following transient exposure to auxin has been reported for auxin action on intact pea roots (Evans, 1976a) and both intact corn roots (List, 1969) and isolated segments of corn roots (Batra et al., 1975).