The pH profile for acid-induced elongation of coleoptile and epicotyl sections is consistent with the acid-growth theory

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Abstract. The acid-growth theory predicts that a solution with a pH identical to that of the apoplast of auxin-treated tissues (4.5–5.0) should induce elongation at a rate comparable to that of auxin. Different pH profiles for elongation have been obtained, however, depending on the type of pretreatment between harvest of the sections and the start of the pH-incubations. To determine the acid sensitivity under in vivo conditions, oat (Avena sativa L.) coleoptile, maize (Zea mays L.) coleoptile and pea (Pisum sativum L.) epicotyl sections were abraded so that exogenous buffers could penetrate the free space, and placed in buffered solutions of pH 3.5–6.5 without any preincubation. The extension, without auxin, was measured over the first 3 h. Experiments conducted in three laboratories produced similar results. For all three species, sections placed in buffer without pretreatment elongated at least threefold faster at pH 5.0 than at 6.0 or 6.5, and the rate elongation at pH 5.0 was comparable to that induced by auxin. Pretreatment of abraded sections with pH-6.5 buffer or distilled water adjusted to pH 6.5 or above gave similar results. We conclude that the pH present in the apoplast of auxin-treated coleoptile and stems is sufficiently low to account for the initial growth response to auxin.

Key words: Acid-extension – Acid-growth theory – Avena (acid-growth) – Elongation growth – pH profile (cell elongation) – Pisum (acid-growth) – Zea (acid-growth)

Introduction

The acid-growth theory (Rayle and Cleland 1970; Hager et al. 1971) states that auxin initiates rapid cell elongation by causing cells to excrete protons. It predicts that solutions with a pH identical to that of the apoplast of auxin-treated tissues should cause tissues to elongate at rates comparable to that induced by auxin, as long as the solutions can penetrate into the cell wall. While exact pH values for the apoplast are not available, it seems generally agreed (Jacobs and Ray 1976; Evans 1985; Brummell and Hall 1987; Schopfer 1989) that the apoplastic pH in the absence of auxin is 5.7–6.5 and in the presence of auxin is 4.5–5.0. It is also agreed that incubation of sections in solutions of pH 5.7–6.5 causes only low rates of cell elongation (Rayle 1973; Cleland 1980; Kutschera and Schopfer 1985), but disagreement exists as to whether incubation in a solution at pH 5 will cause elongation at a rate comparable to that induced by auxin.

Rayle (1973) removed (peeled) the epidermis from Avena coleoptile sections to facilitate entry of buffers and preincubated the sections 60–90 min in a pH-6.8 buffer prior to placing them in acidic solutions. The resulting extension was considerably greater at pH 5 than at pH 6 and comparable to that of auxin. Similar pH profiles were obtained for in-vitro acid-extension of frozen-thawed Avena coleoptiles by Rayle and Cleland (1977) and for in-vivo extension of both maize coleoptile and sunflower hypocotyl sections (Vesper 1985). Kutschera and Schopfer (1985) using maize coleoptiles and Schopfer (1989) with Avena coleoptiles obtained similar results when the abraded or peeled sections were preincubated in a pH-6.5 buffer.

On the other hand, Kutschera and Schopfer (1985) and Schopfer (1989) reported that when sections were preincubated in distilled water after abrasion or peeling, they underwent significant acid-extension only when the pH was lower than 4.5, and that an unphysiologically low pH was required to give elongation comparable to auxin. They suggested that a water pretreatment more closely resembles the in-vivo situation, and that the promotion of growth at pH 5 following pretreatment with neutral buffers is the result of a release of growth inhibition upon removal of the buffers, and not to a growth-promoting effect of the pH-5 buffer itself.

Here we report on pH-profile experiments conducted with Avena and maize coleoptile and pea epicotyl sections which received no pretreatment between the abra-
sation of the cuticle and the start of the pH treatments. This experimental design should eliminate changes in the capacity of the walls to undergo acid-induced wall loosening (CAWL) that could occur during the pretreatment, and should indicate the state of the CAWL in vivo. The experiments described here were carried out in three separate laboratories by seven investigators, with similar results. Data presented in the figures will be identified as to the laboratory in which they were obtained.

Materials and methods

The procedures used in each laboratory will be described separately. Seattle. Seeds (caryopsis) of *Avena sativa* L., cv. Victory (Swedish National Seed Co, Svalöf), were soaked in water for 1 h, then planted in vermiculite, covered, and allowed to germinate in a room at 25 ± 2°C with constant dim red light (< 5 μmol·m⁻²·s⁻¹) to suppress mesocotyl growth. After 4 d, coleoptiles 25–32 mm in length were selected, and abraded with 8–14 strokes of Rottenstone abrasive (Empire White Products, Newark, N.J., USA). After washing, 5-mm sections were cut, starting 3 mm from the tip, measured with a microscope fitted with an eyepiece micrometer, and either preincubated or placed directly in 10 ml of 5 mM K-phosphate buffer, pH 3.5–6.5, in plastic Petri dishes, 60 mm diameter, 15 mm high. The dishes were shaken at 50 rpm in the same red-light room, and lengths were remeasured after 30, 60, and 120 min. Preincubation solutions consisted of distilled water or 5 mM K-phosphate buffer, pH 6.5.

Seeds (caryopsis) of *Zea mays* L., cv. B73 × Mo17 (Brayton Seed Co, Ames, Ia., USA) were germinated and handled in the same way as the *Avena* seedlings, except that 5-d-old seedlings were used to obtain the 25–32-mm-long coleoptiles. Seedlings of *Pisum sativum* L., cv. Alaska (W. Atlee Burpee Co., Warminster, Penn., USA), were grown for 7 d in dim red light. After abrasion, 5-mm sections were cut from 15–40 mm long third internodes, starting 3 mm below the hook.

Values given are the averages ± SE of 5 or 10 sections. All experiments were repeated a minimum of three times. The effectiveness of the abrasion was checked by determining the extent to which 0.1% Neutral red penetrated the epidermal cells in 1 min; in general 70–80% of the epidermis of *Avena* and 40–60% of maize coleoptile sections was open to the external solution.

San Diego. Seeds of *Zea mays* L., cv. B76 × Mo17, were grown as in Seattle, except that the incubation temperature was 23–24°C. After 5 d, sections 12 mm long were cut from coleoptiles approx. 30 mm in length, starting 3 mm from the tip, and abraded with two or three strokes of Rottenstone abrasive on each of the four sides. The sections were then pretreated for 60 min in 5 mM 2-(N-morpholino)ethanesulfonic acid (Mes)-NaOH buffer, pH 6.5, or in distilled water whose pH was adjusted to 6.5 with NaOH. The sections (10/treatment) were then incubated in 10 ml of 5 mM Mes-NaOH buffers, pH 4.0–6.5, and the extension was measured at 30, 60 and 120 min with the aid of a dissecting microscope. *Pisum* and *Avena* seedlings were grown as described above.

Columbus. Seeds (caryopsis) of *Avena sativa* L., cv. Victory (Svalöf) were imbibed in distilled water in darkness for 12 h and then planted in wet vermiculite. The seeds were germinated and seedlings grown in complete darkness for 4–5 d at 26°C. Coleoptiles 15–20 mm in length were selected and a 10-mm section was excised under dim green light, beginning 3 mm from the tip. The primary leaves were removed, the sections were abraded with Rottenstone, and 10 sections were strung on a thin wire with the uppermost section positioned directly under the arm of a position-sensor transducer unit (model 33–34; Metripak, Cleveland, Oh., USA). Total extension of the column of 10 sections was measured by recording the displacement of the transducer arm. Initially, the column of coleoptile sections was held for 1 h in a chamber containing 30 ml of either distilled water (pH adjusted to 6.5) or 5 mM Mes-NaOH buffer, pH 6.5. The pH of the growth medium was monitored and adjusted if necessary with NaOH. The solutions were continuously oxygenated using pure oxygen. The chamber was then drained and 30 ml of either distilled water or 5 mM Mes-NaOH buffer, pH 4.0–6.5, was added in place of the preincubation solutions. Total extension after 30, 60 and 120 min was determined from the extension-versus-time curves.

Results

Previous studies have indicated that rapid acid-induced extension of abraded coleoptile sections occurs at pH 5 and below for sections pretreated in a pH-6.5 buffer (Rayle 1973; Vesper 1985); we have confirmed this for *Avena* and maize coleoptiles and pea epicotyl sections (data not shown). On the other hand, Kutschera and Schopfer (1985) and Schopfer (1989) reported that when abraded coleoptile sections were pretreated with water, rapid acid-extension only occurred at pHs below 5. This, too, we have confirmed for maize (data not shown) and *Avena* coleoptile sections (Fig. 1), using for the pretreatment distilled water without any pH adjustment. The similarity between the pH profiles for water-pretreated sections obtained in this study (Fig. 1, curve B) and that reported by Schopfer (1989; Fig. 1, curve C) demonstrate that differences in methods of abrasion, experimental technique and seed source are unlikely to have affected the results.

*Avena* coleoptile sections incubated in buffers immediately after abrasion elongated rapidly at pH values below 6.0 (Fig. 1, curve A). The elongation rate during the first 30 min in a pH-5.0 buffer was nearly identical to the rate

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Fig. 1. pH profiles for acid-extension of abraded *Avena* coleoptile sections that received no pretreatment between abrasion and incubation in 5 mM K-phosphate buffers at pH 4.0–6.5 or were pretreated for 90 min in distilled water. Curve A, no pretreatment (Seattle); Curve B, pretreatment 90 min distilled water (Seattle); Curve C, pretreatment 2 h in distilled water, redrawn from Fig. 2 of Schopfer (1989). Rates for curves A and B calculated from extension during first 30 min; curve C from maximum extension rate at each pH. *Shaded bars* = auxin-induced growth rate for comparable sections.