Evidence that acid solutions induce plant cell elongation by acidifying the cytosol and stimulating the proton pump

Benno Brummer, Hubert Felle* and Roger W. Parish

Cytology, Plant Biology Institute, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland and **Botanical Institute, Justus Liebig University Senckenbergstrasse 17–21, D-6300 Giessen, FRG*

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Acetic acid (3 mM, pH 4.5) stimulated elongation growth of maize coleoptiles at a much higher rate than citric acid at the same pH and concentration. The effect of these solutions on cytosolic pH and membrane potential of maize rhizodermis cells was measured with microelectrodes. Citric acid caused a decrease in cytosolic pH and a slow membrane hyperpolarization. Acetic acid induced a larger and more rapid cytosolic acidification and membrane hyperpolarization. Hence, the degree of growth stimulation by the acids was positively correlated with the extent of their cytosolic acidification and stimulation of the proton pump. We suggest the acids induce growth by acidifying the cytosol and stimulating the proton pump rather than via direct acidification of the cell wall.

Acid-induced growth *Cell wall acidification* *Cytosolic acidification* *Indoleacetic acid* *Membrane potential* *Zea mays*

1. INTRODUCTION

The discovery that acid solutions could stimulate elongation of seedling stems and coleoptiles lead to the hypothesis that auxins act by acidifying the cell wall [1,2]. This acidification might be achieved by direct or indirect stimulation of the outwardly directed, electrogenic proton pump present in the plasma membrane of plant cells [1–6]. Cell wall acidification is stimulated by auxin treatment, however, there is often a lack of correlation between proton efflux and growth rate [7–11].

We have suggested that the primary effect of auxins is to lower cytoplasmic pH [12]. Subsequent stimulation of the proton pump rather than cell wall acidification would be involved in growth.

Why then do acid solutions stimulate growth? We suspected that they may penetrate the plasma membrane and acidify the cytoplasm. This could explain, for example, why different acid solutions exhibit different pH optima for maximum growth induction [1]. Here, we examine the effects of citric and acetic acids on the growth, cytosolic pH and membrane potential of maize cells. We report a positive correlation between the extent of acid penetration and stimulation of elongation growth.

2. MATERIALS AND METHODS

2.1. *Plant material*

Zea mays (Orla 264) seeds [13] were soaked for 8–12 h in tap water. They were then placed in moist vermiculite in a plastic box for 4–5 days at 25°C in the dark. The apical 3 mm of the coleoptiles were removed, the following 10 mm excised and the primary leaves also removed. The cuticle of the coleoptiles was partially removed by gentle abrasion using wet 400 mesh carborundum (except in the experiment shown in fig.1). The segments were washed with distilled water and buffer.

For membrane potential measurements, roots were excised from 3–4-day-old *Z. mays* (Orla 264) seedlings, washed and fixed in a plexiglass chamber [14]. The measurements were made on the rhizodermis cells.

2.2. *Growth measurements*

Ten coleoptile segments were threaded apical end to basal end onto a stainless-steel wire (0.8 mm diameter), the apical ends facing upwards. The wire with the row of segments was submerged after imbibition for 2 min in vacuo in 27 ml buffer in a test tube. When indoleacetic acid (IAA) was to be

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added (fig.1) a separate tube was prepared and the wire simply transferred. In the experiment where buffers were changed (fig.2), the segments were continually perfused at 7 ml·min^{-1}. At various times the wire was removed and the length of the coleoptile row measured.

2.3. *Measurement of membrane potential*

A standard electrophysiological apparatus was used [13]. Micropipettes were pulled on a Getra instrument (vertical) from fiber glass-filled borosilicate tubing (Hilgenberg) and filled by capillary deplacement with 3 M KCl. Tip diameters were 0.3–0.5 μm. Membrane potentials were recorded from rhizodermis maize root cells (Orla 264) in a plexiglass chamber that was continuously perfused by the test buffer and allowed horizontal approach by the microelectrodes [14].

2.4. *Preparation of the pH-sensitive microelectrodes*

The pH-sensitive microelectrodes were fabricated as in [15] with slight modifications to stabilize the tip against intrusion of cytoplasm [16]. The pipettes were dipped with the blunt end into a mixture of 0.2% dimethyldichlorosilane/benzene and baked at 180°C for 30 min to provide a water-repellent interior surface. For further stabilization of the tips, 0.1% polyvinylchloride dissolved in tetrahydrofuran was sucked into the tip. The proton exchanger resin (Fluka, Buchs, no.82500) was backfilled into the tip, as was the remainder of the capillary with 3 M KCl. These electrodes had resistances of 5–8 × 10^{10} and displayed a slope of 56–58 mV per pH unit between pH 4 and 9.

2.5. *Recording of the intracellular pH*

The pH measurements were carried out on root hair cells of *Z. mays*. The pH electrode always records a sum of membrane potential plus the electromotive force of the pH difference. A second electrode, placed in the same cell, only detects the membrane potential. A high impedance (10^{15}) differential amplifier (WPI, FD 223) recorded and subtracted the signals from the two electrodes, which were monitored on a pen chart (Kontron W+W 314).

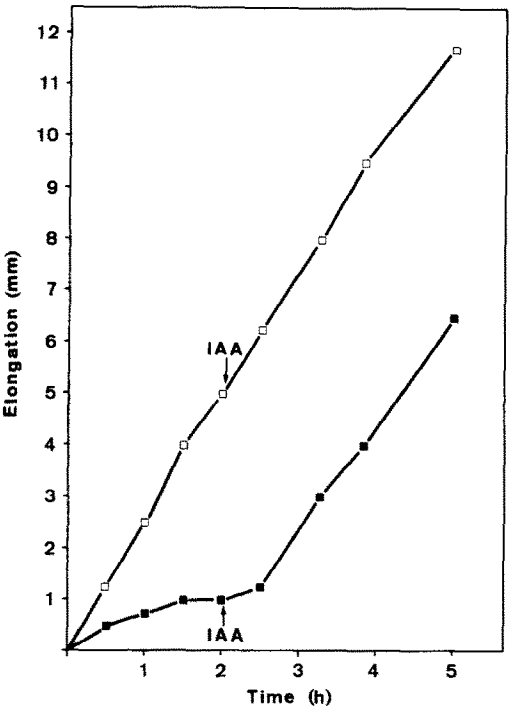

Fig.1. Effect of IAA and acetate on elongation growth of coleoptiles. (□) 20 mM Na acetate–HCl, pH 4.5; (■) 20 mM Na acetate–HCl, pH 7.0. IAA (20 μM) was added after 122 min to both treatments.

3. RESULTS

Elongation growth of coleoptiles was dramatically stimulated in acetate at pH 4.5 as compared to pH 7.0 (fig.1). The addition of 20 μM IAA at pH 4.5 had no further effect on growth. After an initial lag IAA added at pH 7.0 stimulated growth at a similar rate to acetate at pH 4.5 (fig.1).

The relative effects on growth of citric and acetic acids when added at the same pH (4.5) and concentration (3 mM) are shown in fig.2. Citric acid stimulated growth slightly above the rate measured in Na phosphate buffer (pH 4.5). Replacing the citric acid with acetic acid resulted in a dramatic 2–3-fold stimulation of growth.

Since coleoptiles presented technical problems, we measured the effects of the acids on membrane potential using root rhizodermis cells. Perfusion of the roots with citric acid (pH 4.5) resulted in a slow hyperpolarization of 20 mV (fig.3). Acetate was then added and a further hyperpolarization of

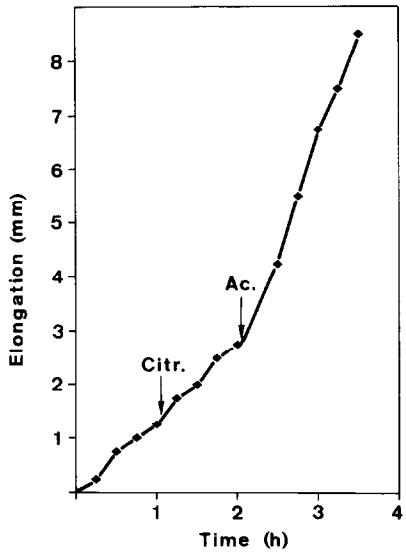

Fig.2. Effects of citric and acetic acids on elongation growth of coleoptiles. Segments were perfused initially with buffer A [10 mM Na phosphate buffer (pH 4.5), containing 1 mM KCl and 0.1 mM $CaCl_2$] at 7 ml·min^{-1}; after 64 min this was replaced by 3 mM citric acid–NaOH (pH 4.5) in buffer A; after a further 60 min citric acid was replaced by 3 mM Na acetate–HCl (pH 4.5) in buffer A.

25 mV rapidly occurred. Hyperpolarization following acetate treatment of oat coleoptiles has been reported [17,18].

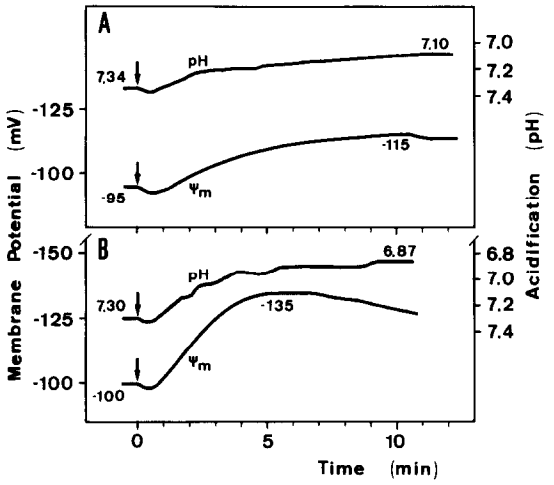

Fig.4. Effects of citric and acetic acids on the cytosolic pH and membrane potential of maize rhizodermis cells. Conditions as in fig.3. (a) 1 mM citric acid–NaOH, pH 4.72. (b) 1 mM Na acetate–HCl, pH 4.8.

These results suggested the acids may be penetrating the plasma membrane and acidifying the cytosol. We tested this possibility by measuring changes in cytosolic pH and membrane potential simultaneously in the same cell (fig.4). When 1 mM citric acid (pH 4.72) was added, the cytosolic pH decreased from 7.34 to 7.20 within the first 3 min and then slowly to 7.10 during the

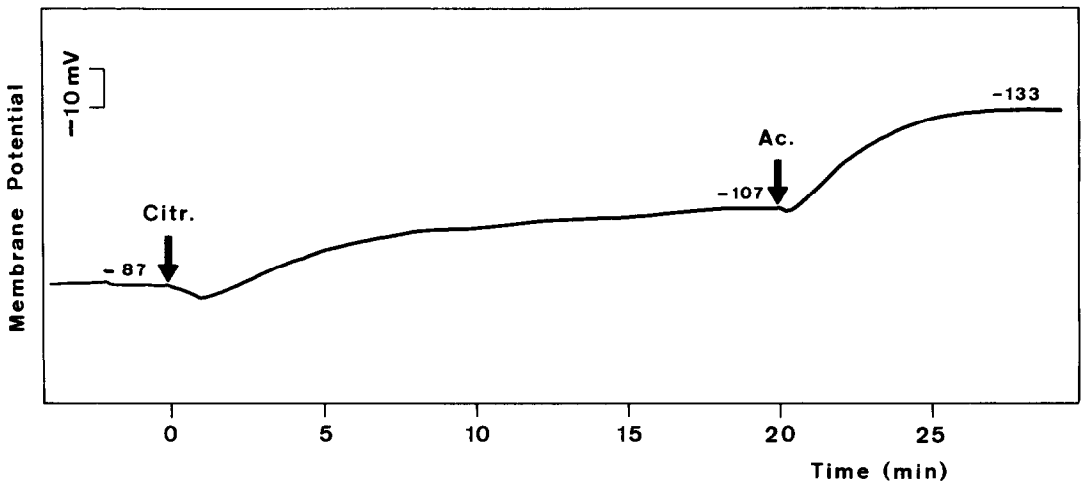

Fig.3. Effects of citric and acetic acids on the membrane potential of maize rhizodermis cells. A root was isolated from a 4–5-day-old seedling, fixed horizontally in a chamber and perfused at 10 ml·min^{-1} with buffer A. At t = 0 2 mM citric acid–NaOH (pH 4.5) in buffer A was added. At t = 20 min citric acid was replaced by 2 mM Na acetate–HCl (pH 4.5) in buffer A. The numbers indicate the potential measured.

subsequent 7 min. A hyperpolarization of 20 mV occurred. Perfusion with acetic acid (1 mM, pH 4.80) led to a more rapid initial decrease in cytosolic pH (0.3 units during the first 3 min). The pH fell from 7.30 to 6.87 during the first 10 min of treatment and then remained stable. A rapid hyperpolarization of 35 mV took place during the first 5 min, followed by a slow depolarization (8 mV during 5 min). This depolarization may reflect changes in membrane leak conductance as reported for *Neurospora* [19].

4. DISCUSSION

The results show that both citric and acetic acids acidify the cytosol and hyperpolarize the membrane of plant cells under conditions where they induce coleoptile growth. Unionized molecules of the weak acids present at the low external pH can penetrate the plasma membrane. In the alkaline cytosol they would dissociate, acidifying the cytosol and stimulating the electrogenic proton pump. The results in fig.3,4 indicate that the penetration of the plasma membrane by citric acid is less than that by acetic acid. Hence, membrane hyperpolarization is slower and less marked. A positive correlation exists between the degree of growth stimulation by these acids and their acidification of the cytosol.

After a lag of some minutes IAA also induces hyperpolarization coinciding with the initiation of net proton secretion [20–22]. We have suggested that a central effect of auxins is cytosolic acidification which then leads to stimulation of the proton pump [12]. Unionized weak bases, such as procaine and NH_3, penetrate the plasma membrane and reionize in the cytosol by gaining protons. Hence, cytosolic pH is raised, pump activity is reduced and depolarization occurs. Such substances inhibit auxin-induced growth [23]. 1-Naphthyl acetate penetrates the plasma membrane and is hydrolyzed by a cytosolic esterase. The subsequent acidification of the cytosol stimulates the pump and induces growth [10]. The carboxylic ionophore monensin leads to acidification of the cell wall when coleoptiles are incubated in alkaline buffer containing Na^+ [24]. Neither pump stimulation nor growth occur even though IAA will induce growth under similar conditions. Under conditions where monensin transports protons into the cytosol, the proton pump is stimulated and growth induced [23,24].

These results support the idea that protons must be excreted via the pump for growth to occur. We have suggested that changes in ψ_m and transmembrane ion gradients resulting from pump stimulation are involved in elongation growth [12]. Nevertheless, pump stimulation may simply reflect cytosolic acidification. Hence, other effects of the acidification rather than pump stimulation (e.g., on metabolism) may be involved in growth. This is difficult to test as it would require uncoupling of cytosolic pH and pump activity. Although inhibition of the pump by vanadate does inhibit growth [25], the result is hard to interpret as the pump is integrated in metabolism.

Following a short period of oxygen deprivation, oat coleoptiles [26,27] and pea stem segments [28,29] showed a temporary increase in growth rate when reimmersed in an aerated solution. During anaerobiosis the pH of the 'cell sap' (the 39000 × *g* supernatant following homogenization of oat coleoptiles in liquid N_2) dropped from 6.3 to 5.9, mainly due to an increase in lactic acid concentration [27]. The ATP level fell around 75% but, following the supply of air, returned within 1 min almost to the original level [27]. Hence, cytoplasmic acidification was again presumably responsible for increased growth, proton pump stimulation first occurring when the ATP had been replenished. The 'burst' of growth following anaerobiosis was partially suppressed by neutral or more alkaline buffers; however, once again different buffer solutions were differently effective [27]. Rice seedlings begin to etiolate under anaerobic conditions, even in the presence of light [30,31].

Whether the anaerobiosis-induced growth has physiological significance may depend on the degree to which ATP levels fall, since a too drastic reduction will prevent the growth response. Nevertheless, the phenomenon may assist plants to grow under conditions of varying oxygen partial pressure and assist the growing regions to reach more favorable conditions [27].

In conclusion, although acid solutions may loosen the wall structure in vitro [1,32], we believe their primary growth-stimulating effect in vivo is cytosolic acidification. Subsequent stimulation of the proton pump may then lead to elongation growth.

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