Calcium and the mechanical properties of soybean hypocotyl cell walls: Possible role of calcium and protons in cell-wall loosening

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Abstract. The role of calcium in the mechanical strength of isolated cell walls of soybean (Glycine max (L.) Merr. cv. Wayne) hypocotyls has been investigated, using the Instron technique to measure the plastic extensibility (PEx) of methanol-boiled, bisected hypocotyl sections and epidermal strips, and atomic absorption spectroscopy to measure wall calcium. Plastic extensibility was closely correlated with the growth rate of intact soybean hypocotyls. Removal of calcium from isolated cell walls by ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) or low pH increased PEx, while addition of calcium decreased PEx; both effects were reversible. The amount of calcium removed and the increase in PEx at pH 4.5 were strongly dependent upon the chelating ability of the buffer anion. There was a direct correlation between the amount of calcium removed from the wall by EGTA or acid and the increase in PEx. Removal of up to 60% of the calcium increased PEx of half-sections up to two fold, but further loss of calcium caused a much greater increase in PEx. With epidermal strips, PEx increased only when calcium was reduced below a threshold. At pH 3.5, there was an additional increase in PEx after a lag of about 2 h; this additional increase may be the result of acid-induced cleavage of a different set of load-bearing bonds. We conclude that calcium bridges are part of the load-bearing bonds in soybean hypocotyl cell walls, and that breakage of these crosslinks by apoplastic acid participates in wall loosening. Acid-induced solubilization of wall calcium may be one mechanism involved in wall loosening of dicotyledonous stems.

Key words: Calcium and cell walls – Cell enlargement – Glycine (cell enlargement) – Hypocotyl – Plastic extensibility

Introduction

The ability of plant cells to enlarge is determined, to a large extent, by the extensibility of their cell walls (for reviews see Cleland 1971; Taiz 1984), which in turn is a function of the spectrum of load-bearing bonds that exist in the walls. Bennett-Clark (1956) suggested that calcium crosslinks between pectic carboxyl groups (calcium bridges) constitute the principal load-bearing bonds in growing cell walls, and that breakage of the calcium bridges might be the mechanism of auxin-induced wall loosening and thus cell enlargement. While addition of calcium to live Avena coleoptile sections inhibited growth (Cooil and Bonner 1956) and decreased tissue deformability (Tagawa and Bonner 1957), studies with isolated Avena coleoptile cell walls (Cleland and Rayle 1977) showed that the plastic extensibility (PEx), as assayed by the Instron technique, was unaffected by conditions that cause the addition or removal of calcium from the walls. It was concluded that calcium bridges are not load-bearing bonds in Avena coleoptile cell walls.

Since then, several authors have suggested that calcium crosslinks might be important in the mechanical properties of the cell walls of dicotyledonous plants. For example, the extension of Helianthus hypocotyl walls was increased by ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (Soll and Böttger 1982), while that of pea epicotyl walls was increased by Mg2+ and...
decreased by Ca$^{2+}$ (Nakajima et al. 1981). No measurements of wall calcium were made on the extending tissue, however, and thus the relationship between wall calcium and wall extensibility could not be determined.

Our research was undertaken to determine the relationship between calcium content and the mechanical strength of soybean hypocotyl cell walls. We used boiled cell walls in order to reduce complications caused by possible enzymatic acid-induced changes in wall extensibility. Since the cuticle is a barrier to the entry and exit of calcium ions and chelators (Prat et al. 1984) even in boiled tissues, bisected sections were employed. The Instron technique was used to evaluate the mechanical strength of the walls. The plastic extensibility, PEx, is a measure of the ability of walls to undergo viscous-extension (Cleland 1967; Fujihara et al. 1978), and is closely correlated with but not identical to the cell-elongation parameter, wall extensibility (Cleland 1967, 1984). It will be shown here that load-bearing calcium crosslinks exist in soybean hypocotyl walls, and it will be proposed that apoplastic acidification may promote wall loosening in these walls by solubilization of this calcium.

Material and methods

Plant material. Seeds of soybean (Glycine max (L.) Merr. cv. Wayne, Champaign County Seed Co, Champaign, Ill., USA) were surface-sterilized in 10% NaOCl for 20 min, then soaked for 1 h in water, planted in wet vermiculite, and the seedlings were allowed to grow at 25±1°C in continuous dim red light (5 μmol·m$^{-2}$·s$^{-1}$; red fluorescent tubes) for 4 d. Hypocotyls of about 3 cm length were used for all experiments. Sections, 20 mm long, and cut starting 2~4 mm below the hook, were preincubated for 1 h in water and were then bisected longitudinally into equal halves with a home-made bisector. In some experiments, epidermal strips were removed from 15-mm half-sections with fine forceps. The bisected sections and the epidermal strips were boiled in absolute methanol for 7–8 min to inactivate enzymes and release the protoplasmic contents. Ca$^{2+}$ in the cytoplasm became absorbed onto the walls during the boiling, as no Ca$^{2+}$ was released to the outside solution (data not shown). Groups of 20 half-sections or 25–30 epidermal strips were then incubated in 20 ml of experimental solutions (unless otherwise stated). The experimental solutions used are described in table headings and figure legends. After incubation, half-sections or strips were washed in water, and stored in fresh methanol until used for wall-extensibility measurements. The pH of each incubation solution was measured at both the start and end of the incubation; if they did not agree within 0.1 pH unit, the treatment was discarded. Plastic tubes were used for all incubations, because it was found that some calcium could leach out from acid-washed Pyrex glass tubes in the presence of EGTA or low pH.

Measurement of plastic extensibility. Wall extensibility was measured by the Instron technique, as described by Cleland (1967). Half-sections were placed between the clamps and extended twice to 60 g load at the rate of 3 cm·min$^{-1}$. This force was selected because it gave a longitudinal stress on the walls comparable to that of turgor (calculations not given). Epidermal strips were extended twice to 20 g. From the slopes of the first and second extensions at 50 g (half-sections) or 16 g (epidermal strips), total, elastic and plastic extensibility values were determined. The values were expressed as tissue plastic extensibility (PEx), with units of percent extension/100 g load, rather than as plastic compliance values. This means that differences in average hypocotyl diameter (such as occurred in the experiments of Figs. 3 and 4) will result in different PEx values, even when the actual plastic wall compliances are the same. The Instron clamps were covered with Parafilm to avoid calcium contamination of the sections.

Calcium measurement. After extension in the Instron assay, the half-sections were oven-dried (70°C for 48 h), weighed, then extracted with concentrated nitric acid for 4 h (1 ml/20 half-sections or 30 epidermal strips), and calcium was measured with an atomic absorption spectrophotometer (Model 303; Perkin-Elmer, Norwalk, Conn., USA). Calcium is expressed as μg (g DW)$^{-1}$ of the walls.

Growth measurements. Groups of ten 10-mm sections were weighed, then incubated in 3 ml of 10 mM K-phosphate buffer, pH 7, +1 mM KCl, ±50 mM CaCl$_2$, +10 μM indole-3-acetic acid or fusicoccin. After 3 h, the sections were reweighed, boiled in methanol, and their PEx was determined. Growth as increase in fresh weight was calculated from the initial and final weights.

Replication. Each datum point is the average for ten half-sections or fifteen epidermal strips. Each experiment was repeated at least three times. Standard errors were calculated for each datum point and were less than 10% unless otherwise indicated. Both PEx and the calcium content of the walls were found to vary, depending upon the exact location along the hypocotyl where the section was cut, and the diameter of the sections, which varied from experiment to experiment. As a result, absolute values from one experiment could not always be compared to those from a different experiment (e.g. Figs. 3, 4). On the whole, however, the experiments were reproducible from one day to another.

Results

The mechanical strength of soybean hypocotyl cell walls was assayed by measuring the plastic extensibility, PEx, by the Instron technique. Although PEx is not identical to wall extensibility (the cellular growth parameter), their relationship is shown by the close correlation between PEx and the growth of soybean hypocotyl sections under a variety of growth conditions (Fig. 1). The plastic extensibility of boiled, bisected soybean hypocotyl sections was altered both by the addition and the removal of calcium (Fig. 2). When the half-sections were incubated in 50 mM CaCl$_2$ +5 mM Na-acetate buffer, pH 6.0, for 3 h, tissue calcium increased by over sevenfold and PEx decreased, relative to tissues treated with buffer alone. When the sections were incubated in buffer with 0.1 mM EGTA for 3 h, wall calcium was re-