Inhibition of Low pH-induced Elongation in *Avena* Coleoptiles by Abscisic Acid

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ABSTRACT

An angular position-sensing transducer was used to make continuous measurements of acid-induced elongation of *Avena sativa* coleoptile segments. Elongation rates at pH 4.5 (5 mM succinate buffer) were about 5-fold greater than those at pH 6.0. Buffered 0.1 mM abscisic acid produced a partial decrease of the growth rate. Pretreatments with abscisic acid buffered at pH 6.0 usually caused a further reduction of the elongation rate when the coleoptile segments were subsequently placed in buffer at pH 4.5 containing abscisic acid. Abscisic acid did not completely prevent the pH effect in any of these experiments, and the brief latent period of the pH response was not affected by abscisic acid treatments. At pH 4.5, where the inhibitory effect of ABA was maximum, low pH-induced elongation was also inhibited by KCN and HgCl2. These results suggest that pH-(4.5) induced elongation in this system may be dependent on some metabolic processes and that abscisic acid-induced inhibition of this elongation may involve an interaction with these processes.

Recent investigations (1, 2, 4) of rapid stimulation of cell elongation by acidic media may provide valuable information in the study of hormone effects on cell wall extensibility. Similarities in the growth kinetics of rapid elongation induced by low pH and auxin have evoked suggestions (4) of possible common mechanisms of action. Went and Thimann (6) concluded in 1937 that low pH promotes growth by activating endogenous auxin. The indication that acid-induced elongation may be insensitive to metabolic inhibitors, which strongly inhibit auxin-promoted elongation, and the fact that the latent period of the auxin-induced response is 8 to 12 min, whereas that for the low pH-induced response is less than one min, suggest that there are some fundamental differences between the two responses (1, 3, 4). In the present study, ABA and metabolic inhibitors were both found to inhibit acid-induced elongation in *Avena* coleoptiles.

MATERIALS AND METHODS

A 1-cm segment was cut from dark-grown 4-day-old *Avena sativa*, L. var. Victory coleoptiles which had been given a 15-min red light treatment on the 3rd day to suppress mesocotyl growth. The primary leaves were removed, and 10 of these segments were strung on a wire and inserted in a measuring chamber in which they were completely immersed in an aerated buffer. The segments were allowed to equilibrate in a succinate buffer, pH 6.0, for 1 hr or longer, after which time a low growth rate was established. The chamber was then quickly drained and refilled with the buffer to be tested. An angular position-sensing transducer was used to produce continuous elongation curves which were analyzed by computer and replotted by an IBM 1130 line plotter as previously described (5).

A 5 mM succinate buffer was selected for use in these experiments, since it consistently maintained the pH required for the various trials. Citrate buffer was also tested but proved less satisfactory, since the citrate at pH 6.0 caused a large initial stimulation of growth during the hour equilibration period before switching to a low pH. An inorganic MOPS2 buffer gave pH responses comparable to succinate but was somewhat more variable, and the pH-induced elongation rates did not remain constant as long.

RESULTS

After 1-hr equilibration in succinate buffer at a pH of 6.0, a low, steady growth rate was established which was not altered by a fresh addition of the pH 6.0 buffer. However, as the pH of the new bathing solution was lowered below 6.0, the elongation rate of the coleoptiles increased (Fig. 1). About a 10-fold increase in elongation rate was observed after a change in pH from 6.0 to 3.2, but the increase persisted for only about 20 min, after which time elongation ceased, and, in some cases, slight shrinkage occurred.

The effects of pH 4.5 were investigated most thoroughly, since this treatment produced about a 5-fold increase in elongation rate which persisted for longer than 1 hr. When 0.1 mM ABA was present in the pH 4.5 buffer, the stimulating effect of the low pH was reduced (Fig. 2). Pretreatment with 0.1 mM ABA at pH 6.0 usually caused further inhibition of the elongation rate, when the coleoptiles were subsequently placed in pH 4.5 buffered ABA. The magnitude of the pH responses and their inhibition by ABA were somewhat variable, but when an entire set of experiments was performed on 1 day with seedlings from the same planting, inhibitory effects were consistently observed with ABA.

The same inhibitory patterns with ABA were found for responses at pH 4.0 and 3.2 even though the pH-induced elongation rates are not constant for the same time period. In no case did ABA completely prevent a growth response to low pH, and the brief latent period (usually less than 1 min) of the pH response was not affected by ABA treatment.

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3 Abbreviation: MOPS: morpholinopropanesulfonic acid, Calbiochem.
This study on the effects of ABA on low pH-induced elongation was undertaken to elucidate the mechanism of ABA action, since it had been reported in the literature (3) that this rapid low pH response was insensitive to metabolic inhibitors. When ABA was shown, in our experiments, to inhibit low pH-induced elongation, it appeared that ABA was acting through a physical wall stiffening process. Protein synthesis was apparently not involved, since pretreatment of the coleoptiles with cycloheximide had no effect on the response to low pH (Fig. 3) in agreement with findings of Rayle and Cleland (4). However, in our subsequent studies, both KCN and HgCl₂ were found rapidly to inhibit pH-induced elongation at pH 4.5 where the effect of ABA was maximal.

When 0.1 mM KCN was tested in the present system with a pH change from 6.0 to 4.5, pretreatments of 0, 30, and 90 min all resulted in inhibition of low pH-induced elongation (Fig. 4). Likewise, 0.1 mM HgCl₂ inhibited pH-induced elongation when added at the time of pH change: long pretreatments with high concentrations of HgCl₂ which might cause damage to cell membranes were avoided. KCN may slightly extend the latent period for the pH effect, and in some cases, a 90-min pretreatment with 0.1 mM KCN eliminated the response to low pH completely.

**DISCUSSION**

Effects of various acid pH values (6.0–3.2) on the rates of coleoptile elongation observed here essentially confirm results of other workers (1, 2, 4).

Although addition of ABA to coleoptile segments growing in the presence of exogenous auxin inhibits elongation with a latent period of about 5 min, the ABA inhibition of the pH response seems to be a slower process. If ABA was added at the time the pH was lowered, significant inhibition of elongation may not be apparent until after 20 or 30 min. Little additional inhibition was found when the coleoptile segments were pretreated for 30 min with ABA at pH 6.0 before reducing the pH to 4.5. However, when the coleoptile segments were given a 90-min pretreatment with ABA, a marked inhibition in the subsequent elongation rate was immediately observable at the beginning of the acid-induced growth. ABA treatments in these experiments did not extend the short latent period for the low pH response.

Evans et al. (1) report CO₂-induced elongation of coleoptiles (which they interpret to be essentially equivalent to that of an acid effect of pH 3.8) to be insensitive to such metabolic inhibitors as KCN, NaF, and HgCl₂. They do, however, report a 40% inhibition in the case of a 1-hr pretreatment with 1 mM
KCN. The apparent discrepancy between the results of Evans et al. and those in the present study wherein low pH-induced elongation is strongly inhibited by KCN and HgCl₂ may be due to the difference in pH employed. Evans et al. used a pH of 3.8 (CO₂-saturated), whereas a pH of 4.5 (succinate-buffered) was used in the present experiments. There was also evidence (not shown) in the present experiments that the inhibitory effect of ABA on low pH-induced elongation was less at pH values below 4.5. D. L. Rayle (personal communication) has also found KCN and HgCl₂ to inhibit low pH responses in several species, and he suggests investigation of the possibility that this inhibitory effect may simply be due to a slight reduction in turgor. Hager et al. (2) report complete inhibition of acid-induced extension of intact cell wall aggregates of Helianthus hypocotyls by Cu²⁺ ions.

The implication from our experiments with KCN and HgCl₂ at pH 4.5 is that there is some metabolic dependence in the rapid elongation promoted by this low pH. Hence, the possibility that the mechanism of ABA-induced inhibition of acid-promoted elongation involves a metabolism-dependent process cannot be ruled out.

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LITERATURE CITED