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Short Communication

Comparative Effects of Hydrogen Ions, Carbon Dioxide, and Auxin on Pea Stem Segment Elongation[1]

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The similarity of growth stimulations by hydrogen ions and auxin has led several workers to propose that auxin action proceeds by the same cell wall loosening process that is brought about by hydrogen ions (3, 5, 9, 13). Cleland (5) and Hager *et al.* (9) suggested that auxin exerts its initial effect on the cell wall by increasing the proton concentration. Extension would proceed as acid-labile, alkali-stable bonds are broken in the cell wall (5, 12). Since the response to hydrogen ion appears to occur in all tissues in which growth may be stimulated with auxin, this suggestion has met with favor. The rapid effect of CO_2 in stimulating growth has also been shown to involve low pH (7, 8), although there is evidence that these effects may be separated (12) and that there is a close link between CO_2 and auxin-stimulated growth (11). In this paper we show that the growth responses of pea stem segments to auxin and CO_2 may be separated from the stimulation by low pH.

MATERIALS AND METHODS

The plant material for experiments with live tissue (*in vivo*) consisted of 7-mm green or etiolated segments cut from the upper portion of the third internode of 7-day-old etiolated or 8-day-old green seedlings of *Pisum sativum* var. Alaska. Seedlings were grown in the dark for 5 days, transferred to continuous illumination (40.3 wm^{-2}; <2% incandescent) and used after 3 days as light-grown (green) tissue. Etiolated seedlings were used after 7 days in darkness. In experiments where red light was given, plants were continuously illuminated for 3 days at a light intensity of 1.1 wm^{-2} with filtered light of 650 nm, after 5 days of darkness. All *in vivo* experiments were performed in darkness at 25 C. Experiments with killed tissue (*in vitro*) were performed on plant material grown in the same manner as outlined above, except that after segments had been cut, the tissue was frozen at −13 C, then thawed in 7.5 mM citrate buffer (pH 6.3) at room temperature prior to use.

The method of measuring growth was adapted from Barkley and Evans (2) and dela Fuente and Leopold (6). Briefly, the procedure was to measure the elongation of a stack of pea stem segments (7 segments) with a Metripak angular-position sensing transducer (6). Segments of peas were stacked in a plastic holder and submerged in continuously recirculating buffer solution (citrate buffer, 7.5 mM) in a glass chamber (2). Oxygen was bubbled continuously through the solution from a syringe needle inserted into the base of the chamber. The sensing axis of the transducer was attached to a 1.5-cm needle, the tip of which rested on a piece of capillary tubing at the top of the stack of pea stem segments. The displacement of the needle by the elongating segments was recorded on a Honeywell recorder (Electronik 194). Displacement of the recorder pen had been calibrated to the displacement of the sensing needle with a micrometer. The growth rate was taken as the slope of the recorder plot and was determined at 2.5-min intervals. Change of pH was obtained by rapidly draining the buffer solution from the chamber and refilling with buffered solution of the desired pH. Treatment with CO_2-saturated buffer or auxin was accomplished in the same manner.

Extension of frozen-thawed segments was measured with an Instron Universal Testing Instrument (TT-C) in the manner described by Rayle *et al.* (13). Tissue was held between clamps held 7 mm apart under a constant load of 20 g. The tissue and clamps were submerged in citrate buffer (pH 6.3) within a Plexiglas chamber. Constant load was applied automatically to the lower clamp, and extension of the tissue was continuously monitored with a position-sensing transducer as the downward movement of the lower clamp assembly.

RESULTS

Effect of Hydrogen Ions and Auxin. The comparative responses to lowered pH and to auxin in both etiolated and green pea stem segments were investigated. Etiolated tissue responded to lowered pH with essentially no lag period and with a duration of about 75 min (Fig. 1, curve A). Tissue grown under red light showed a lesser response to lowered pH (Fig. 1, curve B). Green pea stem segments, however, showed no growth response to lowered pH (Fig. 1, curve C). All three tissues were stimulated *in vivo* by auxin with a 12- to 15-min latent period (Fig. 1, second arrows). The effects of hydrogen ions on etiolated pea stem segments are kinetically similar to those previously reported for coleoptile tissue (7, 12). Green tissue seems unique in that its growth rate is acid-insensitive.

The responses of etiolated and green tissues to lowered pH were also investigated *in vitro* using frozen-thawed pea stem segments. Etiolated segments *in vitro* responded to lowered pH with a rapid initial increase in extensibility (Fig. 2, curve A) similar to the increase in growth rate observed with the same tissue *in vivo*. Green segments, however, showed no increase in extensibility (Fig. 2, curve C). Since green stem segments respond readily to auxin but not to lowered pH, it seems that the response to lowered pH by segments is separate from the effects of auxin, and further that the two responses do not share a common mechanism, as has been proposed for other tissues (12).

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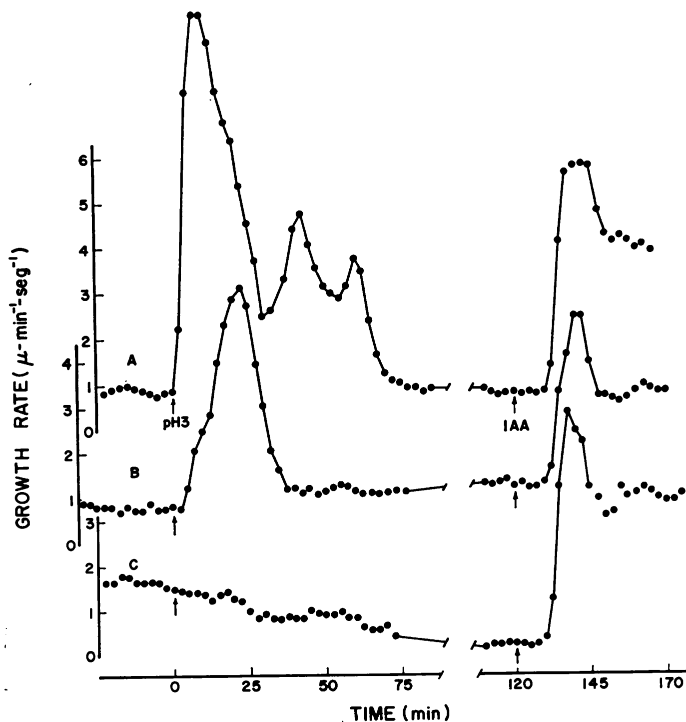

FIG. 1. Effect of low pH on pea stem segments. The growth medium was changed from citrate buffer (7.5 mM, pH 6.3) to citrate buffer (7.5 mM, pH 3.0) at the first arrow, (A–C), followed by an addition of IAA (final concentration 20 μM, pH 3.0) at the second arrow. A: Etiolated segments; B: segments grown under red light (5 days dark followed by 2 days red light); C: light-grown segments.

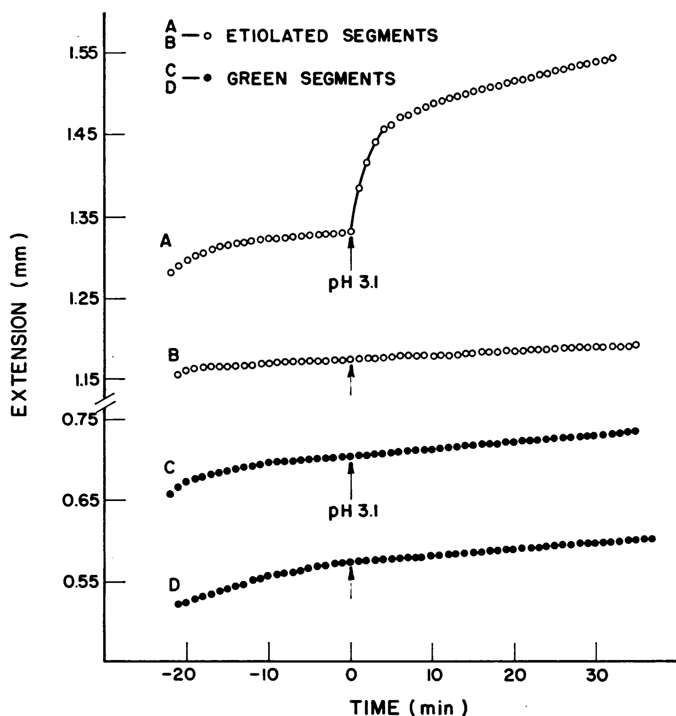

FIG. 2. Effect of low pH on frozen-thawed pea stem segments. At the arrow in curves A and C, frozen-thawed pea stem segment was transferred from buffer at pH 6.3 to buffer at pH 3.0. At the arrow in curves B and D, buffer was changed from pH 6.3 to buffer at the same pH. Extension was measured as the total increase in length in mm. Extension during the 20 min prior to zero time is due to elastic creep.

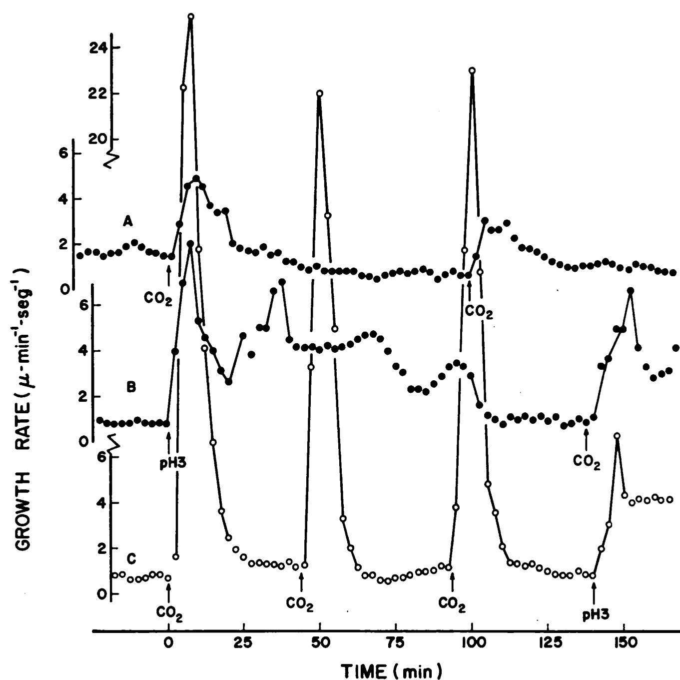

FIG. 3. Effect of CO_2 on pea stem segments. A: Light-grown segments (green), change from citrate buffer (pH 6.3) to CO_2 saturated buffer (pH 4.8) at the first arrow, with a similar change at the second arrow; B: etiolated segments, changed from citrate buffer (pH 6.3) to citrate buffer (pH 3.0) at the first arrow followed by a change to citrate buffer (pH 3.0) saturated with CO_2 at the second arrow; C: etiolated segments, buffer (pH 6.3) to CO_2 saturated buffer (pH 4.8) at the first, second, and third arrows, followed by a change to citrate buffer (pH 3.0) at the fourth arrow.

Effect of Carbon Dioxide and Hydrogen Ions. The growth response to CO_2 was measured by bathing stacks of stem segments in citrate buffer (pH 6.3) and rapidly changing the bathing solution to the same buffer saturated with CO_2 (pH 4.8). An enhancement of the growth rates of green and etiolated segments in response to CO_2-saturated buffer is shown in Figure 3, curves A and C, respectively. The response is very rapid (less than 2 min). Maximum rate is obtained within 5 to 7 min. The duration of the response is 25 to 30 min, about the same timing that is observed for complete removal of CO_2 by continuous oxygenation. The response to CO_2 could be evoked by a second introduction of CO_2-saturated buffer. The second increase in growth rate in response to CO_2 was markedly less in green segments, as has also been shown for etiolated coleoptile sections (8). The response of etiolated segments to CO_2 was five times as large as the response of green segments (Fig. 3, curve C).

The response of etiolated segments to low pH was not changed appreciably by previous exposure to CO_2 (Fig. 3, fourth arrow of curve C). On the other hand, the response to CO_2-saturated buffer was markedly reduced after a previous low pH treatment (Fig. 3, second arrow of curve B). This decrease in responsiveness is likely a separation of the effect of low pH and CO_2 as shown by Rayle and Cleland (12), since the ability of segments to respond to low pH has been depleted by the previous low pH experience.

DISCUSSION

The data reported here show that low pH does not promote elongation in green pea segments, either *in vivo* or *in vitro,* as it does in etiolated tissue. We believe that this difference is not due simply to greening of the tissue, for green segments

from seedlings given red light or intermittent white light still show a response to lowered pH. The condition of unresponsiveness to low pH was obtained when seedlings were exposed to continuous white light. All of the stem tissues tested, etiolated or green, retained the ability to respond to auxin.

Transferring etiolated plants to light increases the amount of cuticular lipids (10) and will probably increase the barrier to penetration. However, the difference in hydrogen ion responsiveness in green and etiolated tissue cannot be attributed to a cuticular barrier to penetration, since removal of the cuticle will not enhance pH responsiveness in green tissue (G. M. Barkley, unpublished data). Also, uptake through cut surfaces would allow for some uptake of hydrogen ions and some response, well within the time course of experiments presented here (Fig. 1, curve C).

A direct effect of hydrogen ion in wall loosening was suggested by Brecht as early as 1936 (4). Only recently has this phenomenon come under close scrutiny (12, 13). Rayle *et al.* (13) have suggested that wall loosening involves a reversible breakage of some acid-labile, alkali-stable cell wall cross-link. It may be that this type of cell wall bond is modified during growth under continuous illumination to an extent that it becomes immune to attack by hydrogen ions. Growth of cotton fibers under constant illumination has been shown to occur with a different pattern of cellulose fibrils (1).

Stimulations of growth by CO_2 were obtained in both etiolated and green pea stem segments, although etiolated segments were markedly more sensitive to CO_2 (Fig. 3, curves A and C). Cleland (5) has suggested, and our results indicate, that a part of a CO_2 stimulation of growth is a consequence of the lowered pH associated with the dissolved CO_2 in the medium. In etiolated segments, a CO_2 stimulation of growth would involve both a response to CO_2 and to the lowered pH associated with it. If etiolated tissue is given a low pH treatment, it is still able to respond to CO_2 (Fig. 3, curve B), and this response is of the same order of magnitude as observed for the green segments (Fig. 3, curve A).

The similarity of CO_2 and auxin in their ability to stimulate growth in green pea stem segments supports the contention by Pope and Black (11) that these two agents share common features in growth stimulation. Evans *et al.* (8) suggested that CO_2 acts by acidification. Our results indicate that at least some of the CO_2 response is separate from the stimulation of growth by low pH.

In conclusion, our comparative experiments with green and etiolated pea stem segments show that, whereas both tissues respond to auxin, those grown under continuous white light do not respond to lowered pH. This was confirmed for the same type of plant material *in vitro* by measuring extension after the cells had been disrupted by freezing. Etiolated peas were stimulated in growth by lowered pH *in vivo* and increased in extensibility *in vitro*. The lack of response by green segments to low pH even though they are responsive to auxin appears to deny the suggestion made earlier by Cleland (5) and Hager *et al.* (9) that auxin stimulation of growth may have its initial effect through an increase in hydrogen ions in the cell wall.

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