Plant Physiol. (1977) 59, 1004-1007

Comparison of Auxin-induced and Acid-induced Elongation in Soybean Hypocotyl[1]

Received for publication May 17, 1976 and in revised form November 29, 1976

LARRY N. VANDERHOEF,[2] TSE-YUAN SHEN LU, AND CYNTHIA A. WILLIAMS[3]
Department of Botany, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Acid-induced growth was compared to auxin-induced growth. After a transient pH 4-induced increase in the elongation rate was completed, auxin could still induce an enhanced rate of elongation in soybean (*Glycine max*) hypocotyl segments. This auxin response occurred both when the medium was changed to pH 6 before auxin addition, and when the auxin was added directly to the pH 4 medium. This postacid response to auxin was persistent, and quite unlike a postacid response to acid, which was again shortlived. One mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7) inhibited the first response to auxin (the first response to auxin being similar to the acid response), but not the second response. This did not appear to be simply a hydrogen ion neutralizing effect, however, since a 50-fold increase in buffer concentration at pH 6 did not inhibit the first response. Decrease in the pH of the external medium, previously shown to occur with excised soybean hypocotyl segments, was not affected by auxin. Furthermore, this pH drop, during which the cells appear to be adjusting their external pH to about 5.4, did not result in an increased rate of elongation. Addition of auxin after the equilibrium pH had been attained did not alter the pH, but it did increase the rate of elongation, eliciting a normal auxin response. It was concluded that hydrogen ions do not mediate in long term auxin-induced elongation in soybean hypocotyl.

The hypothesis that H^+ ions act as a second messenger for auxin-promoted elongation (4, 13, 25) is supported by evidence that coleoptile (3, 8, 10, 20, 25, 26), pea (1, 7, 18, 19), and soybean (29) segments elongate more rapidly at low pH, *Avena* and *Helianthus* wall extensibility is increased at low pH (3, 26), auxin-promoted elongation is preceded by auxin-promoted H^+ ion extrusion in *Avena* (5, 6, 13, 25), pea (15), and corn (15), and certain wall-bound enzymes, glycosidases, have low pH optima (16).

Some experiments, seemingly contrary to the tenets of the hypothesis, have been explained, *e.g.* acid-induced elongation, thought not to occur in green pea segments (1), actually does occur if the epidermis is fractured or removed (7). Other data which do not support the hypothesis are not yet entirely explained: (*a*) Penny *et al.* (21), assuming that the xylem vessels are part of the free space that includes the cell wall (27), have determined that auxin-promoted elongation occurs prior to the pH drop in the cell wall (see refs. 5 and 15 for discussion); (*b*) major differences in auxin- and acid-stimulated cell enlargement exist, *e.g.* auxin, but not low pH, stimulates both radial and longitudinal cell growth in lupin hypocotyl segments (23), and auxin-promoted elongation is not transient, as is acid-stimulated elongation, in *Avena* coleoptile segments (25, 26) and soybean hypocotyl segments (29); (*c*) after acid-stimulated elongation has subsided, auxin is still capable of increasing the rate of elongation in *Avena* coleoptile (10, 25, 16) and soybean hypocotyl segments (29); (*d*) wall-bound glycosidases which have low pH optima, described in support of the hypothesis (16), can be inhibited without inhibiting auxin-promoted elongation in lupin (22) and *Avena* (9); and (*e*) auxin does not affect proton secretion in lupin (21) and soybean (28).

These data have led us to examine the relationship of H^+ ion-promoted and auxin-promoted elongation. The accompanying manuscript demonstrates the lack of an auxin effect on medium pH adjustment (28). The experiments described herein compare acid-induced elongation to auxin-induced elongation, and lead us to conclude that H^+ ions do not mediate in long term auxin-promoted elongation in the soybean hypocotyl.

MATERIALS AND METHODS

Soybean seedlings (*Glycine max* L. Merr. var. Wayne) were germinated in the dark, and the elongating segment of the hypocotyl was excised as described (29, 31). Hypocotyl extension was continuously measured with a linear transducer in an apparatus modified after that reported by Green and Cummins (12), as previously described (29, 30, 32), except that the segments were clamped directly into the growth apparatus and monitored during the preincubation period. When the declining growth rate reached 0.2 mm/hr, the experiment began.

For experiments during which pH was altered (see Figs. 1-3), 1 mM K-phosphate (pH 6) containing 30 mM sucrose, was replaced with 1 mM K-citrate (pH 4) containing 30 mM sucrose, and *vice versa*. Auxin (2, 4-D) was added directly to the growth apparatus to give a final concentration of 45 μM. This auxin, at this concentration, has effects identical to IAA, including induction of medium acidification in *Avena* (25) and a dual elongation response (28-30, 32) in soybean hypocotyl (Lu and Vanderhoef, unpublished).

Each growth curve is the average of five to seven experiments.

RESULTS AND DISCUSSION

First we determined the kinetics of acid-induced elongation, followed by auxin treatment of the same segment. We (29) and others (25, 26) have previously reported that acid-induced elongation is not identical to auxin-induced elongation. Rayle (25), in a report on *Avena* experiments, has stated "The elongation response initiated by auxin can be mimicked, in part, by hydrogen ions . . . *a part* of the elongation response to auxin may involve H^+ secretion" (Rayle's italics). In our report on separa-

[1] Research supported in part by National Science Foundation Grant BMS72-02496, and by the Department of Health, Education and Welfare (Biomedical Sciences Support Grant to the University of Illinois).

[2] To whom correspondence should be addressed.

[3] Present address: Department of Botany, University of Montana, Missoula, Montana 59301.

ble responses to auxin (29), we showed that auxin could increase the elongation rate of excised soybean hypocotyl segments from about 0.2 mm/hr to about 0.5 mm/hr after the acid-enhanced rate of elongation had subsided. That is, the transient nature of the acid response was not because of acid damage. In this previous publication, we reported (ref. 29, Table 3) only the achieved auxin-induced elongation rates (auxin was added after various times of segment incubation at pH 4), rather than rate-time graphs, because we were not yet sure if auxin induced both responses when added after acid treatment. We suspected that the acid treatment may have "exhausted" the first response, and the subsequent auxin treatment would induce only the second response. This seemed possible, since the first response to auxin was quite similar to the acid response. It had been previously reported that acid could not reinitiate an increased elongation rate after the first acid response had subsided (26). We now know, however, that auxin addition, after incubation of the segments at pH 4, induces both responses. This is true, whether the pH is maintained at 4 during auxin treatment (Fig. 1), or changed to pH 6 prior to auxin addition (Fig. 2). This does not, however, rule out the possibility that the acid response and the first response to auxin are similar phenomena, since soybean hypocotyl segments, unlike *Avena* (26), will respond to a second low pH treatment (Fig. 3).

Other data indicate that the acid response may be similar to

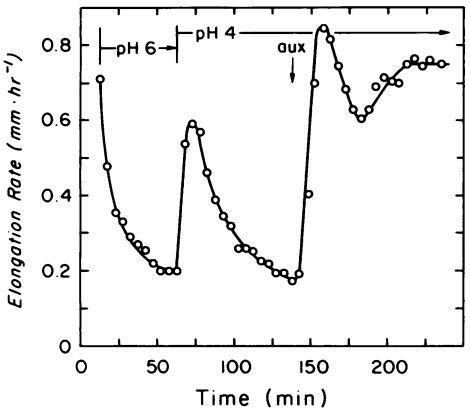

FIG. 1. Auxin addition after acid treatment. A 2-cm elongating hypocotyl segment was excised from a dark-grown 3-day seedling and immediately mounted in the growth apparatus. The initial medium contained 1 mM K-phosphate (pH 6) and 30 mM sucrose. When the elongation rate had declined to the endogenous rate (about 0.2 mm/hr), the medium was changed to 1 mM K-citrate (pH 4) containing 30 mM sucrose. After the elongation rate had again declined to the endogenous rate, auxin was added to give a final concentration of 45 μM.

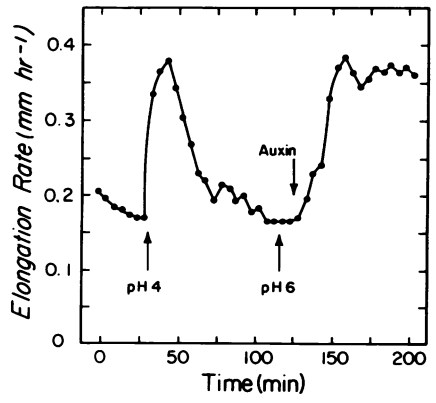

FIG. 2. Auxin addition after acid treatment. Methods were identical to those described in Figure 1 except that the medium was changed back to the phosphate-sucrose (pH 6) buffer prior to auxin addition.

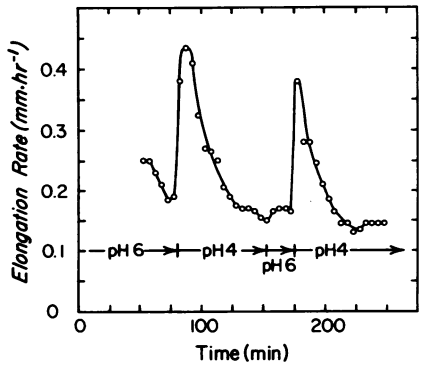

FIG. 3. Postacid acid treatment. Methods and buffers were as described in Figures 1 and 2.

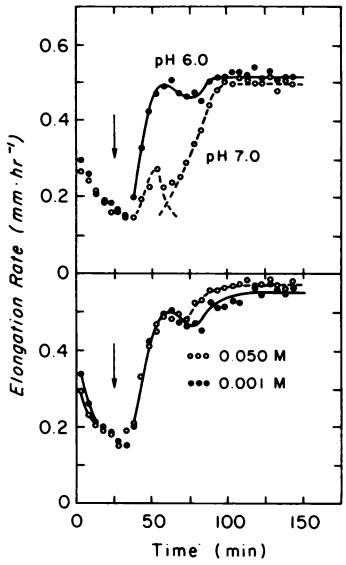

FIG. 4. Selective inhibition of the first response by pH 7 buffer. Bottom graph: KH_2PO_4 (pH 6). Top graph: 1 mM K-phosphate (pH 6), compared to 1 mM HEPES buffer (pH 7). Auxin was added at the arrows. For a discussion of the two elongation responses to auxin, see references 17, 29, 30, 32.

the transient first response to auxin, but is unlike the long term second response to auxin. Ray has stated (24), citing Rayle (25), Hager (13), and unpublished results, that ". . . . induction of elongation by auxin is prevented by sufficiently well buffered media of pH 6 to 8. . . . IAA-induced elongation [can] be suppressed by alkaline media . . . ". While the data of Hager *et al.* are not easily interpreted (the "alkaline medium" of Hager *et al.* was pH 12) experiments show that there is, indeed, an inhibitory effect of supraoptimal pH on auxin-promoted elongation in soybean. It is, however, a selective effect, *i.e.* the first response, but not the second response, is inhibited by 1 mM HEPES buffer (pH 7) (Fig. 4). This effect of pH 7 does not appear to be just a proton-neutralizing effect in the cell wall, since a 50-fold increase in K-phosphate concentration at pH 6 does not inhibit the first response (Fig. 4). Rather, it would seem to be a specific effect of H^+ ion concentration. (To be sure, lower H^+ ion concentrations [pH 7.5–8.5] inhibit the second response as well as the first response. Even at pH 8.5, however, the second response is apparent, achieving a steady elongation rate of about 0.4 mm/hr after 2 hr, while the first response is absent [Lu and Vanderhoef, unpublished].) This selective inhibition by pH 7 HEPES indicates again that the first and second elongation responses are different phenomena (28, 30, 32), but more to the

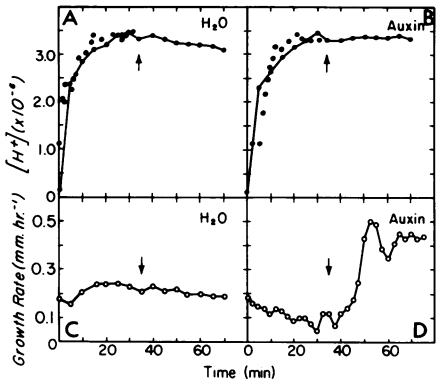

FIG. 5. Effect of pH change and auxin addition on elongation. The natural pH adjustment of the external medium (○; see Fig. 1 ref. 28) was simulated in the growth apparatus by adding acid (●). When the equilibrium pH of about 5.4 was thus artificially attained, auxin (B,D; final concentration, 45 μM) or an equal volume of water (A,C) was added and the growth rate and pH were monitored for the subsequent 50 min.

point here, they indicate that long term auxin-promoted elongation is not mediated by H^+ ion secretion.

We have shown previously that soybean hypocotyl segments readily adjust the pH of the surrounding medium (28). For example, the pH of a weakly buffered solution containing 10 1-cm segments/ml dropped from 6 to 5.5, where it stabilized, in about 20 min. However, this pH change was not affected by auxin addition at the beginning of the experiment, or after the pH of the outer medium had reached its equilibrium value (28). Furthermore, this pH change occurred in the presence of an intact cuticle and epidermis (28).

This cellular adjustment of medium pH can be measured only when a high concentration of segments is present, *e.g.*, about 10 segments/ml in the case of soybean (28) and *Avena* (4, 25). To measure the effect of such cell-mediated pH changes on elongation (where elongation of one segment in 100 ml was measured in the growth apparatus), the pH changes were simulated by adding HCl (Fig. 5). We conclude that in experiments like those in Figure 5, we could adjust the pH in the cell wall with little or no lag time by adjusting the pH in the bathing medium (this was especially likely since the elongation response of soybean hypocotyl segments to lowered pH is almost immediate, ref. 29). Hence, cellular adjustment of medium from pH 6.7 (0.2 μM H^+) to pH 5.5 (3.3 μM H^+), measured with 10 segments/ml (Fig. 5A, 5C) could be simulated in the growth apparatus (1 segment/100 ml) by adding HCl slowly over a 30 min period. When this was done (Fig. 5) it was determined that the acidification did not affect the rate of elongation. When auxin was added at this equilibrium pH of 5.4 ± 0.2 the pH did not change; however, a normal increase in the rate of elongation was measured (Fig. 5 B and D).

Our previous conclusion (28) could now be extended to state the following: soybean segments adjust their medium pH to pH 5.4 ± 0.2; this cellular activity is not affected by auxin; acidification from a pH greater than 5.4 to pH 5.4 ± 0.2 does not affect the rate of elongation; addition of auxin at pH 5.4 ± 0.2, while not affecting the medium pH, will induce a normal elongation response.

CONCLUSIONS

These experiments with soybean hypocotyl do not support the acid growth theory of auxin action. Additionally, lupin hypocotyl segments do not cause a decrease in the external pH in the presence of auxin (21). Auxin induces a pH drop in sunflower, but the effect is cation modifiable, and the lag times for auxin-induced elongation and pH drop are different (ref. 14; see refs. 5, 24, and 25 for discussion). Auxin-requiring sycamore suspension-cultured cells, like soybean hypocotyl segments (27), will lower the external pH, but there is no auxin effect on this process (11).

The acid growth theory of auxin action has attracted much attention recently, primarily because it is a simple theory, and supporting data have accumulated. Negative data are also accumulating, but the theory is new, modification is inevitable, and accurate components of the theory may yet emerge. The fact that there are two separable responses to auxin in soybean hypocotyl may be important in the modification of this, or any, theory of auxin action. The two elongation responses to auxin (29, 34), now known to be biochemically distinct (30), consist of a rapid (lag = 12 min), but transient initial response, and a later (lag = 40 min), long lasting response. Several lines of direct and indirect evidence have made it clear that these two responses, although both expressed as elongation, are different phenomena in soybean (29, 30, 32) as well as cucumber hypocotyl (17). Additionally, it may be relevant that two auxin-binding sites have recently been characterized (2). With regard to the acid growth hypothesis, the activities that constitute the first response may be identical to the activities of the acid response and/or the activities that constitute the first response may induce the activities that constitute the second response.

Note Added in Proof. The results of Figure 4 are only infrequently attained. Furthermore other buffers at pH 7 do not give a similar result. We do not know the reason(s) for this variability. Perhaps this inconsistent character of the first response is related to the inconsistent nature of auxin-induced "H^+ excretion" by *Avena* coleoptile sections (Cleland 1975 Plant Physiol 58: 210–213).

Acknowledgments—The authors thank M. Evans, Ohio State University, and W. Briggs, Carnegie Institute of Washington, Stanford, for their excellent reviews of the manuscript.

LITERATURE CITED

1. BARKLEY GM, AC LEOPOLD 1972 Comparative effects of hydrogen ions, carbon dioxide, and auxin on pea stem segment elongation. Plant Physiol 52: 76–78
2. BATT S, MA VENIS 1976 Separation and localization of two classes of auxin binding sites in corn coleoptile membranes. Planta 130: 15–21
3. BONNER J 1934 The relation of hydrogen ions to the growth rate of the *Avena* coleoptile. Protoplasma 21: 406–423
4. CLELAND RE 1973 Auxin-induced hydrogen ion excretion from *Avena* coleoptiles. Proc Nat Acad Sci USA 70: 3092–3093
5. CLELAND RE 1975 Auxin-induced hydrogen ion excretion: correlation with growth, and control by external pH and water stress. Planta 127: 233–242
6. CLELAND RE 1976 Kinetics of hormone-induced H^+ excretion. Plant Physiol 58: 210–213
7. CLELAND RE, DL RAYLE 1975 Hydrogen ion entry as a controlling factor in the acid-growth response of green pea stem sections. Plant Physiol 55: 547–549
8. EVANS ML 1967 Kinetic studies of the cell elongation phenomenon in *Avena* coleoptile segments. PhD thesis. University of California, Santa Cruz
9. EVANS ML 1974 Evidence against the involvement of galactosidase or glucosidase in auxin- or acid-stimulated growth. Plant Physiol 54: 213–215
10. EVANS ML, PM RAY, L REINHOLD 1971 Induction of coleoptile elongation by carbon dioxide. Plant Physiol 47: 335–341
11. FISHER ML, P ALBERSHEIM 1974 Characterization of a H^+ efflux from suspension-cultured plant cells. Plant Physiol 53: 464–468
12. GREEN PB, WR CUMMINS 1974 Growth rate and turgor pressure. Auxin effect studied with an automated apparatus for single coleoptiles. Plant Physiol 54: 863–869
13. HAGER A, H MENZEL, A KRAUSS 1971 Experiments and hypothesis concerning the primary action of auxin in elongation growth. Planta 100: 47–75
14. ILAN I 1973 On auxin-induced pH drop and on the improbability of its involvement in the primary mechanism of auxin-induced growth promotion. Physiol Plant 28: 136–148
15. JACOBS M, PM RAY 1976 Rapid auxin-induced decrease in free space pH and its relationship to auxin-induced growth in maize and pea. Plant Physiol 58: 203–209
16. JOHNSON KD, D DANIELS, MJ DOWLER, DL RAYLE 1974 Activation of *Avena* coleoptile cell wall glycosidases by hydrogen ions and auxin. Plant Physiol 53: 224–228
17. KAZAMA H, M KATZUMI 1976 Biphasic response of cucumber hypocotyl sections to auxin. Plant Cell Physiol 17: 467–473
18. MARRÉ E, P LADO, FR CALDOGNO, R COLOMBO 1973 Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation. II. Effects

of inhibitors of respiration, oxidative phosphorylation and protein synthesis. Plant Sci Lett 1: 185-192
19. MARRÉ E, P LADO, FR CALDOGNO, R COLOMBO 1974 Correlation between cell enlargement in pea internode segments and decrease in the pH of the medium of incubation. I. Effects of fusicoccin, natural and synthetic auxins and mannitol. Plant Sci Lett 2: 179-184
20. NITSCH JP, C NITSCH 1956 Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight growth test for auxins. Plant Physiol 31: 94-111
21. PENNY P, J DUNLOP JE PERLEY, D PENNY 1975 pH and auxin induced growth: a casual relationship? Plant Sci Lett 4: 35-40
22. PERLEY JE, D PENNY 1974 The effect of some aldonolactones on the IAA-induced growth of lupin hypocotyl segments. NZ J Bot 12: 503-512
23. PERLEY JE, D PENNY, P PENNY 1975 A difference between auxin-induced and hydrogen ion-induced growth. Plant Sci Lett 4: 133-136
24. RAY PM 1974 The biochemistry of the action of indoleacetic acid on plant growth. Rec Adv Phytochem 7: 93-123
25. RAYLE DL 1973 Auxin-induced hydrogen-ion secretion in *Avena* coleoptiles and its implications. Planta 114: 63-73
26. RAYLE DL, R CLELAND 1970 Enhancement of wall loosening and elongation by acid solutions. Plant Physiol 46: 250-253
27. TANTON TW, SH CROWDY 1972 Water pathways in higher plants. II. Water pathways in roots. J. Exp Bot 23: 600-608
28. VANDERHOEF LN, J FINDLEY, J BURKE, W BLIZZARD 1977 Auxin has no effect on modification of external pH by soybean hypocotyl cells. Plant Physiol 59: 1000-1003
29. VANDERHOEF LN, CA STAHL 1975 Separation of two responses to auxin by means of cytokinin inhibition. Proc Nat Acad Sci USA 72: 1822-1825
30. VANDERHOEF LN, CA STAHL, T-YS LU 1976 Two elongation responses to auxin respond differently to protein synthesis inhibition. Plant Physiol 58: 402-404
31. VANDERHOEF LN, CA STAHL, NR SIEGEL, R ZEIGLER 1973 The inhibition of cytokinin of auxin-promoted elongation in excised soybean hypocotyl. Physiol Plant 29: 22-27
32. VANDERHOEF LN, CA STAHL, CA WILLIAMS, KA BRINKMANN, JC GREENFIELD 1976 Additional evidence for separable responses to auxin in soybean hypocotyl. Plant Physiol 57: 817-819