Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3092–3093, November 1973

Auxin-Induced Hydrogen Ion Excretion from *Avena* Coleoptiles

(wall-loosening factor/cell elongation)

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Communicated by K. V. Thimann, July 20, 1973

ABSTRACT ***Avena* coleoptiles excrete hydrogen ions in response to the hormone auxin. Both auxin-induced cell elongation and hydrogen ion excretion occur after only a short lag, and both are prevented by either carbonyl cyanide *m*-chlorophenylhydrazone or cycloheximide. These results support the idea that hydrogen ions act as a second messenger in auxin-induced cell elongation and can be the wall-loosening factor.**

In auxin-induced cell elongation auxin appears to act in one place, the protoplast, while the ultimate effect occurs in a different place, the cell wall (1). A "second-messenger" or wall-loosening factor must connect these events, but its identity has escaped detection. The fact that hydrogen ions can substitute for auxin in causing rapid cell elongation (2–4), and can cause cell-wall loosening in frozen–thawed coleoptile tissues (5) has led to the suggestion that the wall-loosening factor is simply hydrogen ions (1, 3, 5). If so, tissues that undergo cell elongation in response to auxin should also excrete hydrogen ions in response to the hormone. It will be shown here that auxin-induced hydrogen ion excretion does occur in *Avena* coleoptile tissue.

MATERIALS AND METHODS

Plant Material. The plant material consisted of 14-mm sections cut from 25 to 32-mm coleoptiles of *Avena sativa*, cv. Victory. Methods for growing the seedlings and harvesting the sections have been described (6). Since the cuticle is a barrier to both entry and exit of hydrogen ions into the tissue (unpublished data), the coleoptiles were first peeled. This procedure consists of removing the majority of the cuticle and adhering outer epidermal wall in two strips, using fine forceps. The peeled sections grow in response to auxin (data not given).

Methods. Sections were first treated for 30 min in 1 mM K-phosphate buffer (pH 6.0) minus auxin. Groups of 10 sections were then transferred to 15-ml beakers containing 2 ml of the same buffer, ± 2 μg/ml of 3-indoleacetic acid. Since preliminary experiments showed that the hydrogen ion excretion was unaffected by sucrose, the sugar was usually not included in the medium.

Hydrogen ion excretion was monitored in one of two ways. In the first method the pH of the external medium was measured at the start and after 15- or 30-min intervals, with an Instrumentation Laboratory no. 14043 combination pH electrode and a Beckman pH meter. Alternatively, the pH of the solution was adjusted back to 6.0 at 20- or 30-min intervals, with 1 mM KOH or HCl. Treatments were run in duplicate and all experiments were run at least three times.

RESULTS

In the absence of auxin the pH of the medium remains close to 6.0 for at least 150 min, but in the presence of auxin the pH drops rapidly, reaching values as low as 4.7 in 150 min (Fig. 1). The auxin-induced hydrogen ion excretion can be detected after a lag of only 20 min in some experiments (Fig. 2), although the lag is longer in others.

An indication that the appearance of hydrogen ions is due to excretion and not just leakage is obtained from the fact that the auxin-induced appearance of hydrogen ions is prevented by carbonyl cyanide *m*-chlorophenylhydrazone (Table 1) or KCN (data not given). Both of these agents also block auxin-induced cell elongation (data not given). In addition, both the auxin-induced hydrogen ion excretion (Table 2) and cell elongation (7) are inhibited by the protein-synthesis inhibitor, cycloheximide.

DISCUSSION

The role of hydrogen ions in auxin-induced cell elongation has been a matter of conjecture for years (2, 8). Recently Rayle and Cleland (3) and Hager *et al.* (9) independently proposed that hydrogen ions act as a second messenger between the site of auxin action, the protoplast, and the site of wall loosening, the cell wall. The present data provide support for this theory in the following ways.

First, I have shown that *Avena* coleoptiles excrete hydrogen ions in response to auxin. Although hydrogen ion excretion has been demonstrated for various plant tissues including *Avena* coleoptiles (10–13), the effect of auxin on this process has not previously been demonstrated [although the data of Ruge (8) could be interpreted as demonstrating auxin-induced hydrogen ion excretion]. Since similar results have been obtained simultaneously for *Avena* coleoptiles (14), pea epicotyls (15; Cleland, unpublished data), and *Helianthus* hypocotyls (16), it appears that auxin-induced hydrogen ion excretion may be a general phenomenon in tissues that are capable of rapid auxin-induced cell elongation. Furthermore, the hydrogen ion excretion can be detected within 10 min of the time that rapid cell elongation commences. Considering the fact that the hydrogen ions must not only be excreted but must then diffuse into the external medium before they can be detected, it seems likely that hydrogen ion excretion starts simultaneously with or before the onset of cell elongation.

Second, I have shown that agents that interfere with cell elongation also interfere with hydrogen ion excretion. It is not surprising that the respiratory inhibitors carbonyl cyanide *m*-chlorophenylhydrazone and KCN block both processes, but

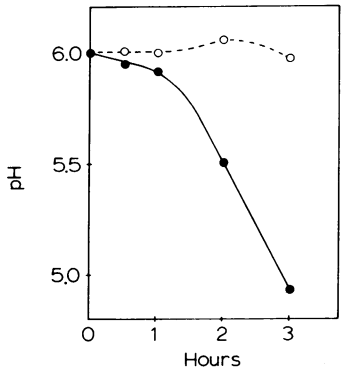

FIG. 1. Auxin-induced H^+ excretion from *Avena* coleoptile tissues. Sections were treated for 30 min in 1 mM K-phosphate buffer minus auxin (pH 6.0) then incubated in the same solutions ± 2 μg/ml of 3-indoleacetic acid. pH was measured at intervals up to 3 hr. Treated with 3-indoleacetic acid (●——●); control, no indoleacetic acid (O- - -O).

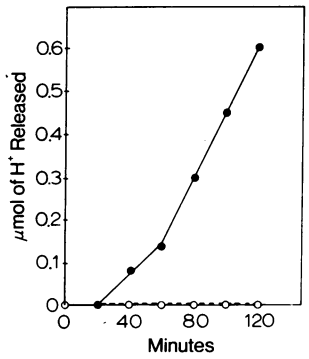

FIG. 2. Rapidity of auxin effect on H^+ excretion. Conditions and symbols were the same as in Fig. 1 except pH was adjusted to 6.0 at 20-min intervals with 1 mM KOH.

the fact that they are both inhibited by cycloheximide is more surprising in view of the lack of detectable side effects of cycloheximide in such tissues (7, 17). These data suggest that the proteins involved in hydrogen ion excretion may be extremely labile, and may be the unidentified growth-limiting proteins (7, 17).

Finally, the amount of the excretion would appear to be sufficient to account for the wall loosening that occurs during auxin-induced cell elongation. Rayle has recently shown (18) and I have confirmed (unpublished data) that a decrease in pH from 6.0 to only 5.0 produces maximal cell-wall loosening in *Avena* coleoptile cell walls. Thus only a slight drop in the pH of the wall solution would appear to be sufficient to account for the cell elongation. My calculations suggest that the magnitude of the auxin-induced hydrogen ion excretion is sufficient to lower the pH of the cell-wall solution to 5 in a matter of minutes.

These results, then, combined with the previous observations that hydrogen ions can replace auxin in inducing cell elongation in intact tissues (2–4) and in causing cell-wall loosening of isolated cell walls (5), provide strong evidence to support the acid-growth theory; namely, that auxin causes

TABLE 1. *Inhibition of H^+ excretion by carbonyl cyanide m-chlorophenyl hydrazone*

Auxin	μmol of H^+ excreted	
	0 CCCP	10 μM CCCP
+	0.70	−0.28
−	0.10	−0.31

Sections were treated with and without 10 μM carbonyl cyanide *m*-chlorophenyl hydrazone in 1 mM K-phosphate buffer (pH 6.0) then transferred to the same solution with or without 2 μg/ml of 3-indoleacetic acid. pH was adjusted to 6.0 every 30 min. Values are for total secretion in 150 min.

TABLE 2. *Inhibition of H^+ excretion by cycloheximide*

Cycloheximide (μg/ml)	μmol of H^+ released per 2 hr	
	+ Indoleacetic acid	− Indoleacetic acid
0	0.65	0
5	0	0.05

Sections were treated for 1 hr in 1 mM K-phosphate buffer (pH 6.0) with or without 5 μg/ml of cycloheximide, then incubated in the same solution with or without 2 μg/ml of 3-indoleacetic acid. pH was adjusted to 6.0 every 30 min. Values are for total secretion in 120 min.

plant cells to excrete hydrogen ions which, in turn, induce cell-wall loosening and thus initiate cell elongation. There is no reason to believe, however, that hydrogen ions are involved in any of the many other auxin effects, some of which [e.g., stimulation of wall synthesis (19)] also may play a role in cell elongation.

This work was supported by an Atomic Energy Commission Contract AT(45-1)-2225-T19. The technical assistance of Donald Marrs is gratefully acknowledged. A report of this work was presented to the Third US-Japan Seminar on Plant Growth Regulation in August, 1972.

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