Plant Physiol. (1974) 54, 213-215

Short Communication

Evidence Against the Involvement of Galactosidase or Glucosidase in Auxin- or Acid-stimulated Growth[1]

Received for publication January 11, 1974 and in revised form March 24, 1974

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ABSTRACT

Research on the mode of action of auxin in the promotion of growth has shown that auxin treatment leads to hydrogen ion secretion and wall acidification. It has recently been reported that auxin stimulates cell wall β-galactosidase activity in *Avena* coleoptiles, presumably by causing cell wall acidification, since the pH optimum for the enzyme is about 5.0. It has been suggested that enhancement of β-galactosidase and/or other glycosidase activity mediates growth promotion by auxin or low pH. This hypothesis was tested by examining the effect of inhibitors of β-galactosidase and β-glucosidase. Severe inhibition of measureable β-galactosidase or β-glucosidase activity was found to have no effect on auxin- or acid-promoted growth. It is concluded that neither β-galactosidase nor β-glucosidase plays an important role in short term growth promotion by auxin or acid. The data do not rule out the possibility that some other cell wall glycosidase is involved in auxin or acid action.

It is well established that hydrogen ions are capable of stimulating elongation to an extent comparable to the stimulation of elongation by auxin (2, 3, 5, 6, 12). This has led to the suggestion that the promotion of growth by auxin may be acid-mediated, *i.e.* auxin may stimulate hydrogen ion secretion causing a reduction in pH within the cell wall to a growth-promoting level (6). This suggestion has received strong support by recent reports that auxin does stimulate hydrogen ion secretion to an extent sufficient to lower cell wall pH to about 5.0, a level capable of causing maximal stimulation of elongation (1, 10, 11).

If it can be established that the action of auxin on growth is largely or entirely due to wall acidification, the question arises as to the cause of cell wall loosening (12, 13) and hence more rapid elongation at low pH. A number of possibilities have been suggested, including the breaking of hydrogen bonds (8), acid hydrolysis of covalent bonds (13), and low pH activation of a wall-loosening enzyme system (6).

Recently, Johnson *et al.* (7) have tested the latter possibility by examining the properties and pH dependence of a number of cell wall bound glycosidases in *Avena*, especially β-galactosidase. They found that the enzyme is inhibited by substances that inhibit the promotion of elongation by acid, and they reported that auxin enhances β-galactosidase activity in coleoptile segments by 36% after a 60-min treatment period. These data suggest the possibility that auxin stimulates growth by stimulating hydrogen ion leakage which activates cell wall glycosidases leading to wall loosening.

If this model of auxin and acid action is correct, it should be possible to greatly reduce the growth response to auxin or acid using specific inhibitors of glycosidases. It is known that the aldonolactones of simple sugars are specific and potent inhibitors of glycosidases active on substrates composed of those sugars (9, and references cited therein). In the work reported here, the effect of galactonolactone and gluconolactone on *in vivo* β-galactosidase and β-glucosidase activity was measured. I found that these aldonolactones strongly inhibit β-galactosidase and β-glucosidase activity but have no effect on growth promotion by auxin or acid.

MATERIALS AND METHODS

Plant Material. Oats (*Avena sativa* var. Victory) were planted in an arrangement similar to that of Wiegand and Schrank (14). They were placed under dim red light for 24 hr and then allowed to grow at room temperature in darkness for 48 hr. Coleoptiles about 3 cm long were selected for harvesting, and segments 10 mm long were cut from them beginning 3 mm from the tip. The leaf was removed from each segment, and the segments were used either for growth tests or enzyme assays.

Chemicals. Indole-3-acetic acid was obtained from J. T. Baker Chemical Co. D-Galactonolactone, D-gluconolactone, *p*-nitrophenyl-β-D-galactopyranoside and *p*-nitrophenyl-β-D-glucoside were purchased from Sigma Chemical Co. All solutions were made fresh just before use.

Enzyme Assays. Enzyme assays were done as described by Johnson *et al.* (7). Twenty coleoptile segments were rinsed with distilled water and placed in a 25-ml Erlenmeyer flask containing 1.5 ml of 13 mM phosphate-citrate buffer with or without 0.1 mM IAA and allowed to incubate at 30 C for 30 min. After 30 min, the assay was initiated by adding 0.5 ml of 40 mM *p*-N-gal[2] or *p*-N-gluc. To those samples in which the effect of aldonolactone inhibitor was to be tested, 0.1 ml of a 0.1 M

[1] This research was supported by National Science Foundation Grant GB37547. Paper No. 862 from the Department of Botany, Ohio State University, Columbus, Ohio 43210.

[2] Abbreviations: *p*-N-gal: *p*-nitrophenyl-β-D-galactopyranoside; *p*-N-gluc: *p*-nitrophenyl-β-D-glucoside.

solution of the appropriate aldonolactone was added at the time of addition of substrate. The samples were then allowed to incubate an additional 30 min at 30 C, and the reaction was terminated by adding 3 ml of 2% (w/v) Na_2CO_3. The absorbance of the solution at 415 nm was then determined using a Bausch and Lomb Spectronic 20 spectrometer.

As noted by Johnson *et al.* (7), the substrates used in this enzyme assay are attacked by exoglycosidases. They point out that, if glycosidases are involved in wall loosening, the active enzyme would most likely be an endoglycosidase not an exoglycosidase. This, plus the probability that *p*-nitrophenylglycosides do not occur in the cell wall suggests that data obtained using these substrates can serve only indirectly as an indicator of cell wall glycosidase activity on endogenous substrates.

Table I. *Effect of Aldonolactone Inhibitors on in Vivo Activity of β-Galactosidase and β-Glucosidase in Avena Coleoptile Segments*

The values shown are averages of four experiments. Data in upper half of table are for β-galactosidase activity on *p*-N-gal. Data in lower half of table are for β-glucosidase activity on *p*-N-gluc.

Treatment[1]		Enzyme Activity	Promotion of Enzyme Activity by IAA		Inhibition of Enzyme Activity by Lactone	
IAA	Lactone		Plus lactone	Minus lactone	Plus IAA	Minus IAA
		A units/20 coleoptile segments	%			
−	−	0.491 ± 0.029				
+	−	0.516 ± 0.034	3.9	5.1	43.5	43.0
−	+	0.280 ± 0.027				
+	+	0.291 ± 0.011				
−	−	0.820 ± 0.013				
+	−	0.872 ± 0.003	15.2	8.7	74.0	75.0
−	+	0.197 ± 0.015				
+	+	0.227 ± 0.010				

[1] IAA: 0.1 mM; lactones: 5 mM; substrate: 30 mM.

Growth Measurements. Elongation was measured using the growth-recording apparatus described in reference 4. For most growth experiments, ten 10-mm coleoptile segments were used, and the growth medium was 2.6 mM phosphate-citrate buffer (pH 6.0) to which appropriate additions of hormone or inhibitor were added. In growth experiments with auxin solutions to which gluconolactone was added, it was noted that at the concentration of lactone used (5 mM), the pH of the medium was reduced to 3.8 by the acidic lactone. (This did not occur in enzyme assay experiments since a 5-fold higher final buffer strength was used.) Therefore the pH of solutions containing gluconolactone was brought back to pH 6.0 with sodium hydroxide just before adding the solution to the chamber. The controls for such experiments were done using auxin solutions that were first acidified to pH 3.8 with acetic acid and then readjusted to pH 6.0 with sodium hydroxide just before use.

The effect of lactone inhibitors on the acid-growth response was investigated by treating the segments first with water and then with citrate buffer at pH 3.8 with or without 5 mM lactone inhibitor. In order to test the possibility that the failure of lactones to inhibit the acid-growth response was due to poor

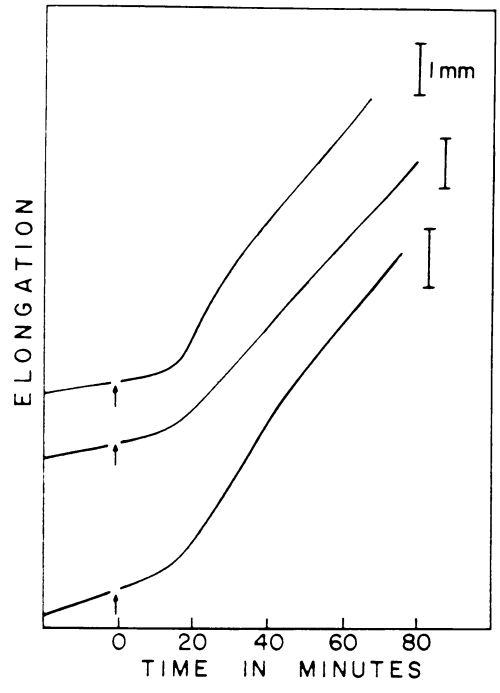

FIG. 1. Effect of galactonolactone and gluconolactone on growth promotion by IAA. Medium changed from 2.6 mM phosphate-citrate buffer (pH 6.0) to 10^{-5} M IAA (upper curve) or IAA plus 5 mM galactonolactone (middle curve) or IAA plus 5 mM gluconolactone (lower curve). The bracket at the end of each curve represents elongation of 1 mm for the entire column of ten 10-mm segments in that experiment.

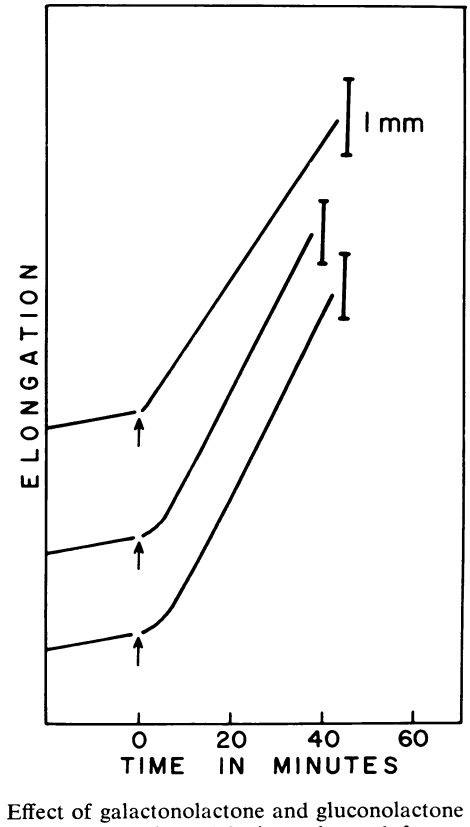

FIG. 2. Effect of galactonolactone and gluconolactone on growth promotion by hydrogen ions. Medium changed from water to 1 mM citrate buffer (pH 3.8) containing no inhibitor (upper curve), 5 mM galactonolactone (middle curve), or 5 mM gluconolactone (lower curve). The bracket at the end of each curve represents elongation of 1 mm for the entire column of ten 10-mm segments in that experiment.

penetration through the cuticle by the inhibitor, acid-growth experiments were also done using peeled (epidermis removed with fine forceps) coleoptile segments. In these experiments, the segments were treated first with 1 mM phosphate buffer (pH 6.0) and then with 1 mM phosphate-citrate buffer, pH 4.6, with or without lactone inhibitor.

The possibility that the failure of the lactone inhibitors to affect the auxin response was due to poor uptake of the inhibitor was also tested. Short (4 mm) coleoptile segments were used in lieu of peeled segments for these experiments. It is reported (7) that the substrates *p*-N-gal and *p*-N-gluc penetrate at least 1 to 1.5 mm into the cut surface of *Avena* coleoptile segments so that, by using 4-mm segments, the majority of the cells should be readily accessible to the even smaller lactone inhibitor molecule.

RESULTS AND DISCUSSION

Table I summarizes the results of experiments testing the promotion of β-galactosidase and β-glucosidase activity by auxin and the inhibition of such enzyme activity by the respective aldonolactones. The stimulation of β-galactosidase activity by auxin reported by Johnson *et al.* (7) was found in all experiments. Although the promotion was consistently seen, it was quite small, averaging 5.1%, *i.e.* about the same magnitude reported by Johnson *et al.* for 30-min pretreatments with auxin. I also observed a small (average 8.7%) but consistent increase in β-glucosidase activity in the presence of IAA.

The inhibition of β-galactosidase activity and β-glucosidase activity by galactonolactone and gluconolactone is strong and consistent both in the presence and absence of IAA. Galactonolactone (5 mM) was found to inhibit β-galactosidase activity on 40 mM *p*-N-gal about 43%, while gluconolactone inhibited β-glucosidase activity on *p*-N-gluc about 75%.

If auxin promotes growth by stimulating β-galactosidase or β-glucosidase activity, these aldonolactone inhibitors should also strongly inhibit the promotion of growth by auxin. Figure 1 shows that this is not the case. The response of *Avena* coleoptile segments to IAA in the presence of either aldonolactone is similar to the response to IAA alone. Pretreatment with the lactone inhibitors for 1 hr was also found to have no effect on a subsequent response to auxin in the presence of inhibitor. Similarly, the lactone inhibitors were found to have no effect on the auxin response in short (4 mm) coleoptile segments used to facilitate uptake of the inhibitors.

Figure 2 shows that galactonolactone and gluconolactone are also without effect on the acid-growth response in *Avena* coleoptile segments. The increase in growth rate upon transfer from water to citrate buffer (pH 3.8) containing 5 mM of either lactone is the same as the increase upon transfer from water to buffer alone. Pretreatment for 1 hr with inhibitor also had no effect on the acid growth response. The acid-growth response was also tested using peeled coleoptile segments as described under "Materials and Methods." Although the acid-growth response in peeled segments was observed to be less strong than in nonpeeled segments, the lactone inhibitors were again found to have no effect, indicating that lack of inhibition is probably not due to poor uptake of the inhibitors.

CONCLUSION

The strong growth-promoting activity of auxin in the presence of inhibitors that greatly reduce measureable β-galactosidase or β-glucosidase activity indicates that these enzymes do not play an important role in growth promotion by auxin. Similarly, the lack of effect of these glycosidase inhibitors on acid-promoted growth indicates that neither β-galactosidase nor β-glucosidase mediates the growth-promoting action of hydrogen ions.

These results, of course, do not rule out the possibility that growth is mediated by some aldonolactone-insensitive cell wall endogalactosidase or endoglucosidase or by another type of glycosidase such as α-mannosidase, which is also reported to have a pH optimum in the acid range (7).

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