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Promotion of Xyloglucan Metabolism by Acid pH[1]

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ABSTRACT

Like indoleacetic acid, buffers of acidic pH, which stimulate elongation of pea (*Pisum sativum* var. Alaska) stem tissue, induce the appearance within the tissue of a water-soluble xyloglucan polymer that probably arises from previously deposited wall material. Neutral pH buffers, which inhibit the elongation response to indoleacetic acid in this tissue, inhibit indoleacetic acid-induced increase in soluble xyloglucan. The findings provide further evidence that release of soluble xyloglucan from the cell walls of pea results from the biochemical action on the cell wall that is responsible for wall extension. The data also indicate that treatment of tissue with either auxin or acidic pH has a similar biochemical effect on the cell wall. This is consistent with the H^+ secretion theory of auxin action.

The acid secretion theory of auxin action, postulated by Hager *et al.* (10) and Cleland (2), proposes that auxin acts by stimulating an outwardly directed H^+ pump in the plasma membrane, which acidifies the cell wall. The lower pH then directly, or by activating a low pH-activated enzyme, breaks bonds in the cell wall to allow wall extension and therefore cell elongation. Evidence for this theory is that acidic media stimulate elongation in auxin-responsive tissues (1, 10, 17, 19, 22); that neutral or alkaline buffers inhibit auxin-induced elongation (5, 10, 18); and that auxin induces release of H^+ ions from coleoptiles (3, 7, 18) and from pea stem tissue (15, 16).

According to the acid secretion theory, the biochemical mechanism underlying wall extension should be the same during treatment with either acidic pH or with auxin (20). Johnson *et al.* (12) have proposed that cell wall-bound hydrolases having acidic pH optima are responsible for wall extension induced by acidic pH. However, known hydrolases do not seem to be responsible for wall extension (8, 21). No direct evidence regarding the chemical effect of acid pH (in the range that induces extension) on the cell wall has yet appeared.

Labavitch and Ray (13) discovered that a specific early effect of auxin on pea stem tissue was to cause release, in water-soluble form, of xyloglucan, presumably derived from the cell walls. They considered that this release might be attributable to the biochemical process that causes wall extension. We have now examined the effect of acid pH treatment and find that it causes a similar release of soluble xyloglucan.

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MATERIALS AND METHODS

The experiments followed the pulse-chase protocol previously described in detail (13). Segments 8 mm long were cut from the elongating third internode of 7-day-old etiolated pea seedlings (*Pisum sativum*, var. Alaska). Before cutting the segment, the internode was gently scrubbed by rubbing it between thumb and forefinger with No. 305 emery powder (Edmund Scientific Co., Barrington, N. J.), suspended in water as a thin paste, to abrade the cuticle (6). The emery was then washed away with water. The segments were depleted of endogenous substrates by a 1-hr incubation in water, labeled during a 1-hr incubation in uniformly labeled ^{14}C-glucose (10 μCi/ml), and then treated for 3 hr in 50 mM unlabeled glucose to dilute radioactive metabolites from internal substrate pools. Media containing 50 mM unlabeled glucose, 17 μM IAA, and/or phosphate-citrate buffers of various pH (in all cases 10 mM, in both K phosphate and Na citrate) were then supplied for an experimental treatment period, the nature and duration of which varied from experiment to experiment as described below. These procedures were conducted at 23 to 25 C.

In experiments in which the effect of acid pH was tested, the pretreatment media contained glucose as described, but the buffer treatment media contained no glucose. This was done in order to make osmotic conditions as favorable as possible for elongation. Table II shows that eliminating glucose from the final treatment media did not prevent the detection of xyloglucan release in response to IAA.

At the termination of treatment, the samples were chilled over ice and ground in ice water. Cell wall material was collected by centrifugation for 2 min at 1000g and was washed twice with cold water. Protein was precipitated from the combined 1000g supernatants using 5% trichloroacetic acid. The supernatant was lyophilized and made 80% in ethanol at 2 C to precipitate water-soluble polysaccharides.

For the experiments in Table I, these polysaccharides were hydrolyzed in 1 N H_2SO_4 for 6 hr at 100 C and passed through Dowex-1 (acetate form) to remove SO_4^{2-} and uronic acid. Sugars were washed through with water, lyophilized, and separated by descending chromatography. Chromatograms were scanned and the radioactivity of eluted peaks was determined by scintillation spectrometry as described previously (13).

For experiments in Table II and Figures 2 and 3, ethanol-precipitable, water-soluble polysaccharides were redissolved in water and passed through Dowex-1 acetate. Neutral components were washed through with water, then acidic components were eluted with 6 N acetic acid. Except for Figure 2, these fractions were concentrated by lyophilization and their radioactivity was determined by scintillation spectrometry. For Figure 2, neutral and acidic fractions were hydrolyzed and their neutral sugars separated by chromatography as described above.

Each radioactivity value is the mean for duplicate samples of 30 pea stem segments.

Elongation experiments followed the same incubation and

Table I. *Effect of IAA and pH on Neutral Water-soluble Xyloglucan*

Xyloglucan was assayed as ^{14}C in xylose and glucose after complete hydrolysis of the water-soluble polysaccharide fraction of the tissue. Data show ^{14}C in samples at the end of the pretreatment period (initial) and after 7 additional hr of treatment. Numbers in parentheses are average deviations of duplicate samples from the mean. Buffer, when present, was 10 mM phosphate-citrate.

Treatment	Glc	Xyl	Elongation[1]
	cpm		%
Experiment 1			
Initial	10,550 (550)	6,810 (950)	
Unbuffered medium	6,730 (120)	4,690 (420)	4.8
Unbuffered + IAA	17,680 (20)	12,780 (80)	29.8
pH 7 buffer	7,130 (220)	5,780 (500)	3.7
pH 7 buffer + IAA	6,960 (1,060)	5,550 (800)	7.4
Experiment 2			
Initial	15,250 (1,320)	7,460 (80)	
pH 7 buffer	12,480 (370)	6,180 (1,090)	6.4
pH 4.5 buffer	16,460 (1,680)	9,070 (690)	12.7

[1] Elongation during 7 hr, as percentage of initial length.

Table II. *Effect of IAA and pH on Soluble Xyloglucan during 2 hr*

Xyloglucan was assayed as ^{14}C in water-soluble neutral polysaccharide. Numbers in parentheses are average deviations of duplicate samples from the mean. Buffer, when present, was 10 mM phosphate-citrate.

Treatment	^{14}C in Soluble Xyloglucan
	cpm
Initial	17,960 (620)
H_2O	12,260 (200)
IAA, unbuffered	21,770 (150)
pH 7 buffer	11,510 (90)
pH 7 buffer + IAA	14,760 (240)
pH 4 buffer	28,610 (230)

treatment protocol described above. At the termination of treatment, the aggregate length of 10 segments from each replicate of each treatment was measured by placing the segments end to end along a guide mounted above a millimeter ruler (9, 11).

RESULTS

In order to induce elongation of etiolated pea stem segments with acidic buffers, it was necessary to abrade the cuticle, which apparently provides a barrier to the entry of buffer substances (4, 22) as it does to H_2O (6). Therefore, segments scrubbed with a thin paste of emery powder were used to test whether acid pH causes a release of soluble xyloglucan similar to that reported for auxin treatment by Labavitch and Ray (13, 14). This release was sought using segments whose cell walls had first been labeled by feeding the segments ^{14}C-glucose for 1 hr, and whose internal pools of wall precursors had then been extensively diluted by incubation for 3 hr in 50 mM unlabeled glucose media before treatment with test media.

We demonstrated, as shown in the upper part of Table I, that IAA treatment of scrubbed segments causes an increase in ^{14}C-labeled water-soluble xylose and glucose polysaccharides comparable to that found previously with unscrubbed segments (13). Table I shows that despite the several hours of pretreatment in a high concentration of unlabeled glucose to dilute precursor pools, IAA can cause the amount of soluble ^{14}C-labeled xylose and glucose polysaccharides to increase well above the initial level present at the time IAA is administered. Because the only known source of ^{14}C-labeled xyloglucan is the insoluble xyloglucan of the cell wall, this indicates, in agreement with the previous conclusion (13), that auxin is causing insoluble cell wall xyloglucan to become released within the tissue in water-soluble form.

Experiment 1 of Table I shows further that 10 mM phosphate-citrate buffer, pH 7, completely prevents release of xyloglucan in response to IAA. This conforms with the fact that buffering at pH 7 virtually prevents the elongation response of scrubbed segments to IAA (Table I, and ref. 18).

As shown in Experiment 2 of Table I, treatment with pH 4.5 buffer induces an increase in ^{14}C-labeled xyloglucan compared with a pH 7 control. The amount released by pH 4.5 treatment in a 7-hr experiment was substantially less than that released by treatment with IAA. The elongation response of this tissue to acidic media differs markedly from the response to IAA. Whereas elongation continues throughout 7 hr in IAA, it virtually stops by about 2 hr in a pH 4 medium (Fig. 1). Therefore, further tests were conducted using just 2 hr of treatment with either IAA or pH 4 medium.

As shown in Table II, pH 4 treatment for 2 hr releases fully as much xyloglucan, compared with a pH 7 buffer control, as unbuffered IAA treatment does in comparison with a water control. It was necessary to use unbuffered IAA solutions here because, as shown above, buffering at neutral pH eliminated both the elongation and the xyloglucan release caused by IAA. The initial pH of the distilled H_2O and the unbuffered IAA solution used in these experiments was 5.3 and 4.8, respectively. It is clear from data of Figure 3, to be discussed below, that the effect of this IAA solution could not have been the result of its pH alone.

The top portion of Figure 2 shows the composition of the water-soluble, neutral ^{14}C-labeled polymer released at acid pH, and assayed by the procedure used in Table II and the experiments subsequently described. It consisted mainly of xylose and glucose, with about 13% of galactose. This agrees with previous results on the composition of the polysaccharide that is released by IAA and which is known to be a xyloglucan (14).

Figure 3 shows how elongation and release of xyloglucan during 2 hr vary with pH between 7 and 3. They increase in parallel down to pH 4, but below pH 4 elongation falls while xyloglucan release remains at a maximum. The data also show that acidic

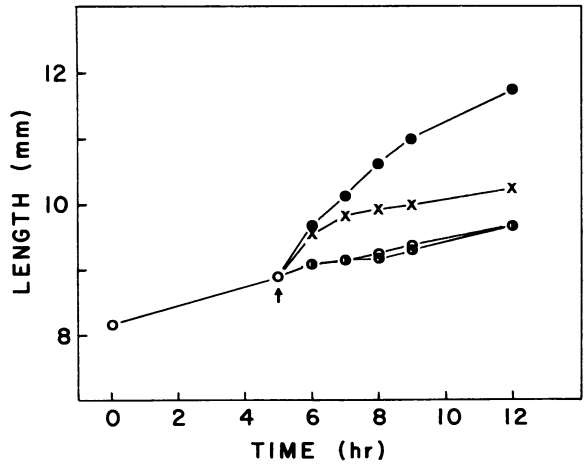

FIG. 1. Changes in tissue segment length over the course of a typical turnover experiment. After 5 hr pretreatment, IAA (17 μM), pH 4 buffer, or control media of water or pH 7 buffer, were added (arrow). IAA: (●); pH 4 buffer: (×); water control: (○); pH 7 control: (◑).

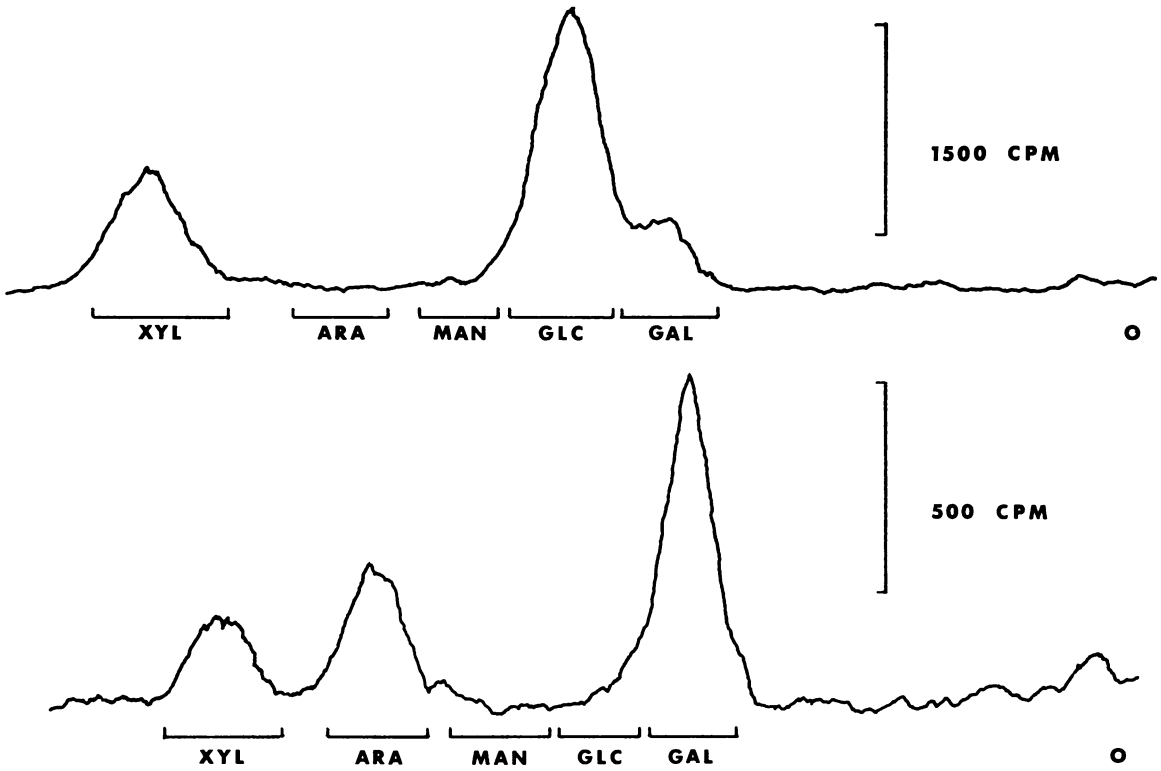

FIG. 2. Scans of radiochromatograms of neutral sugars obtained by hydrolysis of neutral (upper scan) and acidic (lower scan) water-soluble polysaccharides. O indicates origin.

pH, like IAA (13), does not cause a substantial release of water-soluble acidic polysaccharides. This material was composed of galacturonic acid and, as shown in the lower part of Figure 2, galactose, arabinose, and xylose, and may be regarded as pectic polysaccharide. The level of ^{14}C-labeled, soluble pectic polysaccharide actually fell substantially under treatment with pH 3.

DISCUSSION

This work demonstrates that when buffers of acidic pH induce elongation of pea stem tissue, water-soluble xyloglucan becomes released from the cell wall just as it does under treatment with IAA. Furthermore, release of xyloglucan in response to IAA is inhibited by neutral pH buffers just as is the elongation response to IAA. Previous results prove that release of xyloglucan is not caused by the occurrence of elongation (14). Therefore, the present findings add substantial further weight to the conclusion (14) that xyloglucan release reflects or results from the biochemical action on the cell wall that causes the wall to undergo extension growth.

The results indicate that treatment with either auxin or acid pH causes a similar biochemical effect on the cell wall. This is as expected from the hypothesis that auxin acts by causing secretion of H^+ ions into the cell wall space (2, 10), but of course does not prove this hypothesis because apparently similar biochemical changes could be brought about by different mechanisms.

Acid pH probably releases xyloglucan by activating an enzyme with an acidic pH optimum rather than by splitting of "acid-labile" bonds. We infer this because release of xyloglucan does not rise further when the H^+ concentration is raised 10-fold between pH 4 and 3, whereas the rate of an H^+-catalyzed reaction would be expected to increase markedly. That elongation falls as the pH is lowered below 4, whereas xyloglucan release does not fall, can probably be attributed merely to cell membrane damage and resultant loss of turgor at very low pH. The results

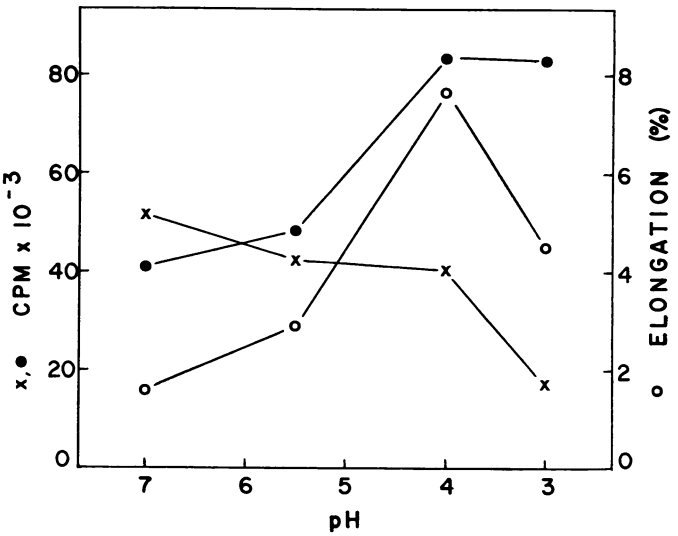

FIG. 3. Effect of pH on radioactivity in soluble xyloglucan and pectic polysaccharide, and on segment elongation. Measurements were made after 2 hr of treatment with buffer media. Elongation is expressed as percentage of initial length (length at time of addition of buffer media). Soluble xyloglucan: (●); soluble pectic polysaccharide; (×); elongation: (○).

at pH 3 provide an additional piece of evidence that xyloglucan release is not being caused by elongation.

The data show that both acid-induced elongation and acid-induced xyloglucan release in pea stem tissue treated according to our protocol are essentially completed within 2 hr of exposure to low pH. This suggests that the acid pH-activated enzyme presumed to be responsible for these effects is relatively short-lived in the cell wall. In contrast, IAA-induced elongation and

xyloglucan release continue for at least 7 hr (14). This difference between acid and auxin effects suggests that auxin may bring about elongation in pea at least partly by a mechanism other than inducing H^+ secretion, for example, by causing transport of the xyloglucan-releasing enzyme from the cell into the cell wall.

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LITERATURE CITED

1. Bonner, J. 1934. The relation of hydrogen ions to the growth rate of the *Avena* coleoptile. Protoplasma 21: 406–423.
2. Cleland, R. 1971. Cell wall extension. Annu. Rev. Plant Physiol. 22: 197–222.
3. Cleland, R. 1973. Auxin-induced hydrogen-ion excretion from *Avena* coleoptiles. Proc. Nat. Acad. Sci. U.S.A. 70: 3092–3093.
4. Cleland, R. and D. L. Rayle. 1975. Hydrogen ion entry as a controlling factor in the acid-growth response of green pea stem sections. Plant Physiol. 55: 547–549.
5. Dolk, H. E. and K. V. Thimann. 1932. Studies on the growth hormone of plants. I. Proc. Nat. Acad. Sci. U.S.A. 18: 30–46.
6. Dowler, M. J., D. L. Rayle, W. Z. Cande, P. M. Ray, H. Durand, and M. H. Zenk. 1974. Auxin does not alter the permeability of pea segments to tritium-labeled water. Plant Physiol. 53: 229–232.
7. Durand, H. and D. L. Rayle. 1973. Physiological evidence for auxin-induced H^+ secretion and the epidermal paradox. Planta 114: 185–193.
8. Evans, M. L. 1974. Evidence against the involvement of galactosidase or glucosidase in auxin- or acid-stimulated growth. Plant Physiol. 54: 213–215.
9. Evans, M. L. and P. M. Ray. 1972. Inactivity of 3-methyleneoxindole as mediator of auxin action on cell elongation. Plant Physiol. 52: 186–189.
10. Hager, A., H. Menzel, and A. Krauss. 1971. Versuche und Hypothese zur Primärwirkung des Auxins beim Streckungswachstum. Planta 100: 47–75.
11. Hashimoto, T. 1959. Effects of light on the indoleacetic acid-induced growth of *Pisum* plant. Sci. Pap. Coll. Gen. Educ. Univ. Tokyo (Biol. Part) 9: 235–254.
12. Johnson, K. D., D. Daniels, M. J. Dowler, and D. L. Rayle. 1974. Activation of *Avena* coleoptile cell wall glycosidases by hydrogen ions and auxin. Plant Physiol. 53: 224–228.
13. Labavitch, J. M. and P. M. Ray. 1974. Turnover of cell wall polysaccharides in elongating pea stem segments. Plant Physiol. 53: 669–673.
14. Labavitch, J. M. and P. M. Ray. 1974. Relationship between promotion of xyloglucan metabolism and induction of elongation by indoleacetic acid. Plant Physiol. 54: 499–502.
15. Marré, E., R. Colombo, P. Lado, and F. Rasi-Caldogno. 1974. Correlation between proton extrusion and stimulation of cell enlargement. Effects of fusicoccin and of cytokinins on leaf fragments and isolated cotyledons. Plant Sci. Lett. 2: 139–150.
16. Marré, E., P. Lado, F. Rasi-Caldogno, and R. Colombo. 1973. 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. 1: 179–184.
17. Nitsch, J. P. and 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.
18. Rayle, D. L. 1973. Auxin-induced hydrogen-ion secretion in *Avena* coleoptiles and its implications. Planta 114: 63–73.
19. Rayle, D. L. and R. Cleland. 1970. Enhancement of wall loosening and elongation by acid solutions. Plant Physiol. 46: 250–253.
20. Rayle, D. L., P. M. Haughton, and R. Cleland. 1970. An *in vitro* system that stimulates plant cell extension growth. Proc. Nat. Acad. Sci. U.S.A. 67: 1814–1817.
21. Ruesink, A. W. 1969. Polysaccharidases and the control of cell wall elongation. Planta 89: 95–107.
22. Yamagata, Y., R. Yamamoto, and Y. Masuda. 1974. Auxin and hydrogen ion actions on light-grown pea epicotyl segments II. Effect of hydrogen ions on extension of the isolated epidermis. Plant Cell Physiol. 15: 833–841.