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Extensibility and rheology of collenchyma cells: II. Low-pH effect on the extension of collocytes isolated from high- and low-growing material

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Low-pH effects were studied on the extension of isolated fresh, methanol-killed and frozen-thawed collocytes. Fresh and frozen-thawed samples responded to low pH. This response decreased with increasing differentiation. A yield stress was found for frozen-thawed samples. The significance of the response during growth and differentiation is discussed.

Creep relaxation parameters have been reported for young and senescent collocytes from the petiole of *Apium graveolens* (*5*, *13*). For better understanding of collocyte wall extension, both growth and differentiation patterns as related to the phyllotaxy of the petioles and their age should be analyzed. At least three parameters can be discussed: longitudinal growth and length of collocytes, and the weight of their wall fraction. Collocyte length was reported to increase with cell wall thickness during differentiation; while growth decreased at the end of differentiation (*21*). Collocytes and epidermal cells were also suggested to be characterized by passive growth (*19*). Consequently, in elongation analysis, an external force can be applied to the collocytes in order to mimic the tension due to the other tissues. Recently, others have observed, when using isolated epidermis of *Avena* coleoptile and *Pisum* stem that frozen-thawed and fresh tissues responded to low pH by a time dependent extension very similar to the response of intact coleoptiles while methanol-killed segments did not (*2*, *3*, *23*).

The pH effect on extension may be characterized by a similar short time response in both in vitro (frozen-thawed tissue, external force applied) and in vivo assays (fresh tissue, turgor pressure as the extending force) (*17*). For peeled *Avena* coleoptile, maximal stimulation in the elongation has been found at pH 5 in both in vivo and in vitro expermients (*18*), while the optimal growth response of peeled segments of *Pisum* was at pH 3.5 for both in vivo and in vitro assays (*2*, *25*). We note here that the xyloglucan metabolism can be changed by auxin and low pH; the xyloglucan level increased in a cold water extract of the wall fraction (*6*, *8*, *9*).

The experiments described below were carried out to understand better the action of low pH on the wall extension of collocytes in relation to their growth and differentiation.

Materials and methods

Plants of *Apium graveolens* were cultivated in a greenhouse with controlled temperature and humidity. When the free part (no leaf lamina) of the external petioles (Fig. 1) measured 22±2 cm (it was designated as the "basal part" of the petiole), plant was used for the experiments.

For growth analysis, India ink marks were made at 5-mm intervals on the basal part. After 10 days, the elongation of each zone was measured and expressed in % of the initial length.

Collocyte bundles were prepared as previously reported (*5*). In the present study, fibers (2 to 3 cm long) were removed from the top of the basal part. Seven to eight fibers were obtained from each petiole. They were immediately placed in 20 ml of buffered solution (citric acid 0.01 M-disodiumhydrogenophosphate 0.02 M) and separated according to position of the basal part.

To measure length, the collocytes were incubated for 10 hr in a pectinase mixture 0.4% pecinol (Röhm, Germany) 16% saccharose. The solution was then vigorously shaken and the length was determined microscopically. Similar observations were done using 12% chromic oxide.

For extension experiments, fresh, frozen-thwaed and methanol-killed collocytes were used. Frozen- thawed collocytes were prepared as follows: the samples were

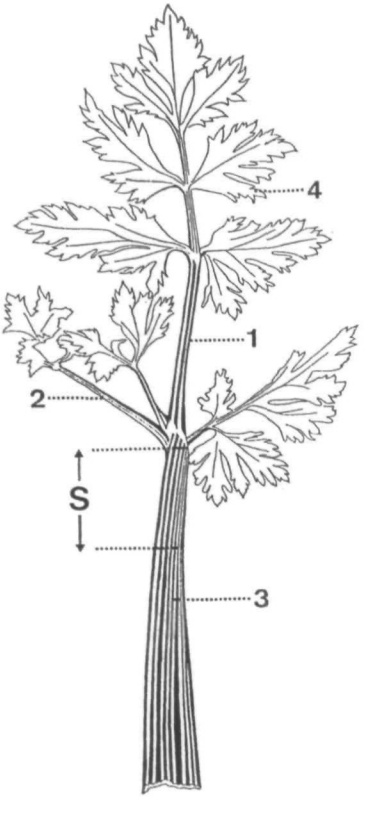

Fig. 1. *Diagram showing a leaf of* Apium graveolens *and the material used in the present assays.* Primary and secondary (1, 2) nervures; free part of the petiole (3); lamina (4); the 3-cm segment(s) employed for the experiments.

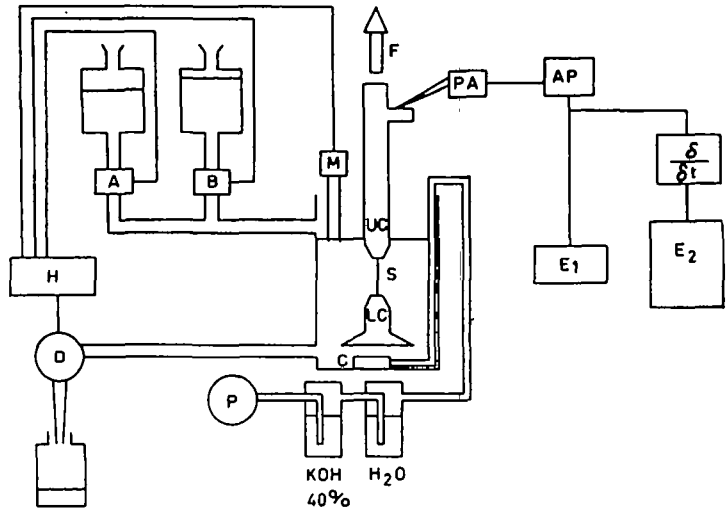

Fig. 2. *Diagrammatic presentation of the instrument used for testing collocyte extension in relation to low-pH effects (see text).*

quickly frozen and kept at −20°C; before use, they were thawed at room temperature and washed with buffer solution. The methanol-killed collocytes were prepared by incubation for 5 min in boiling methanol. The extension of the isolated collocytes was traced with a creep apparatus slightly modified (Fig. 2) from that described previously (*5*). The sample was inserted between a lower (LC) and an upper clamp (UP) attached to a pivoting beam. The collocytes were stretched by a force (F) applied on the other side of the beam by hanging a weight or using a dynamometer. Extension of the fiber caused rotation of the beam which was detected by a TESA transducer (PA), amplified (AP) and transmitted to a Metrohm recorder (E 1). For some measurements, a differential function of extension was required; a differentiator ($\delta/\delta t$) was used after the amplifier and another (E 2) was used for only the extension rate. To analyze the fresh material, an air pump (P), followed by two washing bottles (40% KOH and distilled water) was connected to a porous stone (C) which passed air through the liquid in fine bubbles. Solution changing was controlled by an automatic clock system (H) which switched on filler pumps (A or B) and a draining pump (D). The regularity of the liquid level was controlled by contact electrodes (M).

Creep determinations were performed as previously reported (*5*). External force F=10 g was applied to frozen-thawed specimens (T=1 min).

The weight of the wall fraction (expressed as length unit g/cm) was estimated using a standard method (*1*, *12*). The tension applied was calculated by dividing the force applied (F) by the weight of the wall fraction, assuming that the cell wall density was about 1 (*1*, *11*); the tension was expressed in dynes/cm^2.

In the extension experiments, the force applied (F) was 8 g for fresh collocytes and it was changed for frozen-thawed and methanol-killed specimens. Each extension result represents the measurement of at least 10 collocyte bundles repeated twice. For growth and differentiation data, the values were obtained using at least four plants.

Results and discussion

Young petioles are small and situated in the inner part of the plant. Differentiated petioles are long and located on the exterior. The petioles arranged on a spiral may represent a growth and differentiation gradient for collenchyma. Growth of the basal part of the petiole and the weight of the wall fractions of the collocytes (Fig. 3), length of collocytes and total strain ε_{tot}[1] (Fig. 4) are given as functions of the relative length L_r of the basal part of the petiole:

$$L_r = L_p / L_{max} \qquad (I)$$

L_p: length of the basal part of the petiole

L_{max}: length of the basal part of the tallest petiole of the plant

The relative length of the basal part is a more usuable value than the range of location. The basal parts with a small L_r are located on the top of the celery shoot and are arranged higher on the spiral than parts with a greater L_r. The significant growth of this basal part seems to occur in the 3 cm just below the first nervure. No dramatic gradient of growth is apparent in this zone. As indicated in Fig. 3, growth decreased when L_r increased, while the weight of the wall fractions increased when the growth was low. Fig. 4 shows that collocyte length increased with increasing L_r, but total strain ε_{tot} decreased when L_r was enhanced. Similar results were previously reported for fresh tissues (*5*).

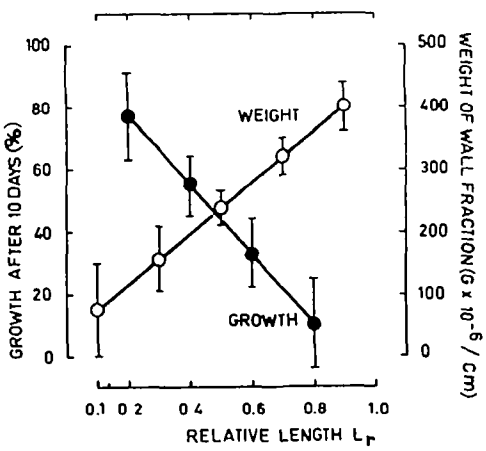

Fig. 3.

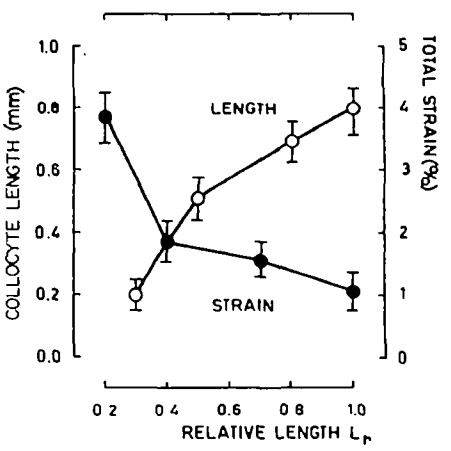

Fig. 4.

Fig. 3. *Relationships between the growth of the 3-cm basal part (just below the first nervure) of the petiole, the weight of the wall fraction of collocytes prepared from the same region of the petiole and the relative length L_r of the basal part.* Measurements were taken after 10 days of growth. Linear regression of the experimental data is reported. Points and vertical lines represent the mean value for a sample ± the confident interval (P = 0.05).

Fig. 4. *Relationships between the length of the collocytes of the 3-cm basal part, the total strain ε_{tot} of frozen-thawed collocytes ($F = 10$ g) and the relative length L_r.* Points and vertical lines represent the mean value ± the standard error.

[1] For critical discussion of this parameters, see Ref. *5*.

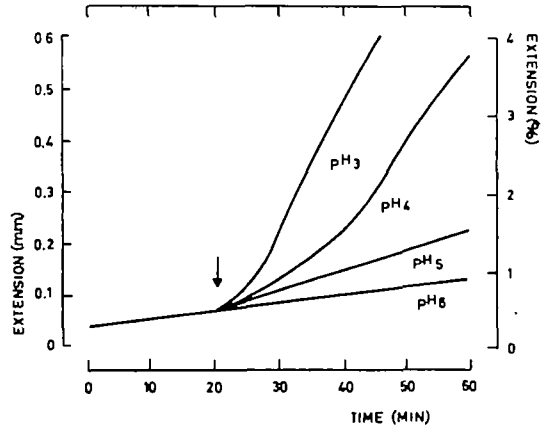

Fig. 5. *The pH effect with time on the extension (absolute and relative values) of isolated fresh collocytes prepared from high-growing material ($F=8$ g).*

Our data clearly show that the basal part with an L_r of 0.1 to 0.2 is high-growing material while the basal part with an L_r of 0.8 to 1.0 is low-growing material.

Fig. 5 shows that low pH induced extension of isolated fresh collocytes of high-growing material with a lag period of 1–2 min. The response of fresh tissues increased with decreasing pH. When pH was changed from an acidic to a neutral value, the extension stopped rapidly; this extension was reversible up to a second extension with an acidic pH (Fig. 6). Other buffers (acetate, glycine) have also been tested and similar results were obtained.

To express the extension rate of collocytes for pH_n, the following equation can be proposed:

$$ER_n = dL/dt \cdot 1/L_o \text{ (expressed in } \% \times 10^{-4}/\text{min}) \quad \text{(II)}$$

dL/dt: elongation rate for a collocyte bundle.

L_o: initial length of the collocyte bundle.

Thus, ER_5, ER_4 and ER_3 are the extension rates of collocytes in buffered solutions of pH 5, pH 4 and pH 3, respectively. The extension rate was measured 10–20 min after the change of solution or when the extension was at a steady state. The pH effect on the extension rate can be easily expressed:

$$\text{at pH 5: } \Delta ER_5 = ER_5 - ER_6 \quad \text{(III)}$$

$$\text{at pH 3: } \Delta ER_3 = ER_3 - ER_6 \quad \text{(IV)}$$

When calculating the Δ ER values, the precision limit of the instruments used was $1\% \times 10^{-4}$/min. When a logarithmic scale was used, the zero value of Δ ER would be transformed in $1\% \times 10^{-4}$/min. As already reported, the pH 3 responses could include a different behavior of extension from that obtained for pH 5 (24).

Fig. 7 shows the effects of pH 5 and 3 on L_r. The decrease of the pH responses was correlated with the growth of basal part (see Fig. 3). Note that the same drop was observed for the relative value for both pH 3 and 5 in relation to L_r. However, for $L_r=1.0$, the effect of pH 3 was tenfold that of pH 5, which was minimal. Such a difference could be related to the decrease of turgor pressure of the collocytes for treatment at low pH.

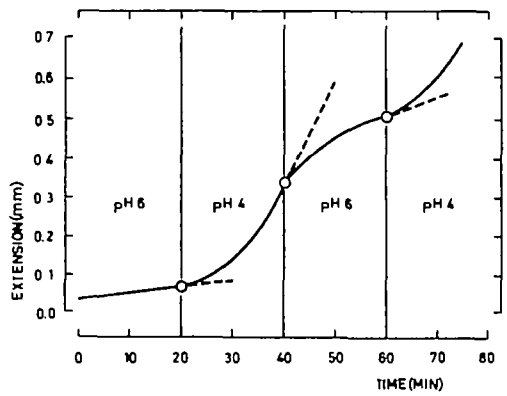

Fig. 6.

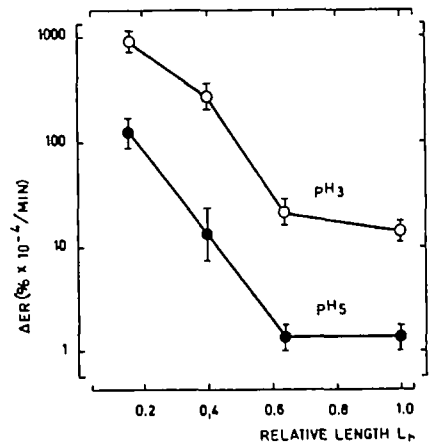

Fig. 7.

Fig. 6. *Effect of pH changes on the extension (absolute values) of isolated fresh collocytes prepared from high-growing petioles (F=8 g).*

Fig. 7. *The pH effect on the extension rate (± standard error) of isolated fresh collocytes in relation to relative length L_r (F=8 g).*

The data of Table 1 support this explanation. Frozen-thawed collocytes behaved like fresh collocytes and the extension rate did not significantly differ except for pH 3 for low-growing collocytes. In such case, they exhibited a lower response than fresh collocytes. It has to be noticed that they lost turgor pressure and that biochemical activity, i.e., the ability to synthesize new cell wall material, was reduced (*17*). However, some enzymes (glucosidases and galactosidases) seem protected against this kind of treatment (*23*).

Methanol-killed collocytes exhibited a very small response to low pH; this was already observed for epidermic cells (*24*). But the decrease of their extension

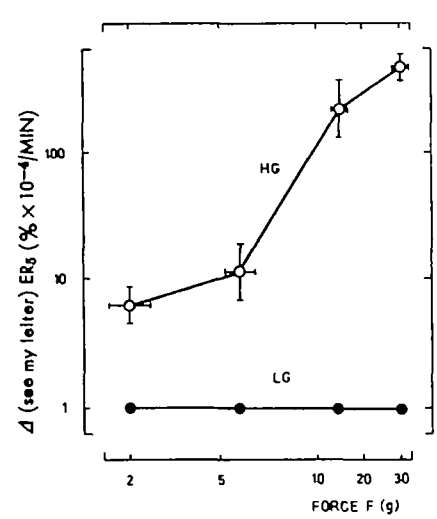

Fig. 8. *The pH effect on the extension rate (± standard error) of frozen-thawed collocytes prepared from high-growing (HG) and low-growing (LG) material.*

Table 1 *pH Effects on fresh (I), methanol-killed (II) and frozen-thawed (III) collenchyma bundles prepared from high-(HG) and low-growing (LG) petioles at a force of 10 g*

	Relative length of the basal part of the petiole (L_r)		pH 5 Effect in $\% \times 10^{-4}$/min log (ΔER_5)		pH 3 Effect in $\% \times 10^{-4}$/min log (ΔER_3)	
	HG	LG	HG	LG	HG	LG
I	0.2	1.0	2.40±0.10[a]	0.00	2.95±0.10[a]	1.20±0.20[a]
II	0.2	1.0	0.88±0.15	0.00	1.58±0.30	0.00
III	0.2	1.0	2.24±0.23	0.00	2.83±0.11	0.00

[a] Standard deviation given for each value.

ability was (when reported in relative values) identical for both pH 3 and 5. In fact, the methanol-killed samples lost most of their protein content in boiling methanol (*24*). Thus, the cell wall materials may have been modified by such drastic treatment.

Most tissues (*17, 23*) show a yield stress for the pH effect on extension when frozen-thawed. Frozen-thawed collocytes of high-growing material displayed similar characteristics (Fig. 8). For low F, only small values were found for Δ ER_5.

In contrast, collocytes of low-growing material showed no significant response. This could be explained by the fact that the cell wall is thicker than in high-growing collocytes. The pH effect on collocytes from low- and high-growing materials to the same force or stress is given in Table 2. Stress large enough to induce a pH effect on collocytes of high-growing material had no effect on collocytes of low-growing material. This could be correlated to a change of the cell wall which takes place at the end of the differentiation and which must be in connection with a decrease of permanent strain ε_p.[2] Some observations also indicate that rheological parameters have been related to an increase of cellulose content in the cell wall (*7*). Other studies on collenchyma cell wall have shown that the increase of differentiation was followed by a large accumulation of cellulose (*20*). Such data could very well explain, at least partially, the present results.

Table 2 *pH Effects on force and stress in frozen-thawed collocytes prepared from high-(HG) and low-growing (LG) petioles*

Force or stress applied		Total strain in % ε_{tot}	Strain ratio $\varepsilon_{el}/\varepsilon_{tot}$	pH Effect in $\% \times 10^{-4}$/min	
				log (ΔER_5)	log (ΔER_3)
Force = 10 g	HG	3.50±0.30	0.45±0.04	2.42±0.10	2.94±0.11
	LG	1.15±0.30	0.72±0.04	0.0	0.0
Stress = 13.4×10^6 dynes/cm^2	HG	3.60±0.30	0.45±0.04	2.42±0.10	2.94±0.11
	LG	2.40±0.45	0.72±0.04	0.0	0.0

Standard deviation given for each value.

[2] This parameter has been critically discussed for a similar material in Ref. *5*.

Conclusion

Growing collocytes exhibit a similar low-pH response to that of epidermal cells. Such responses to pH 3 and 5 do not differ, except in the intensity of the extension. The response to low pH decreased when the growth decreased at the end of differentiation. It has been previously reported that NAA induces increase in both the length of collocytes and their extensibility (*14*) and that NAA acts on the cell wall by decreasing the pH as IAA does (*10*, *16*). At the end of growth, the wall changes from a low-pH sensitive into a low-pH nonsensitive one (*22*). In addition, experiments on low-growing material have shown that the isolated collocytes are not sensitive to NAA (unpublished results). However, it has been noticed that pH-sensitive tissues do not necessarily respond to auxin (*4*). In contrast, fusicoccin induces growth by decreasing the pH of the wall (*10*) and by acting also on the balance between endogenous IAA and ABA (*14*, *15*). Such pH and growth interactions could probably be better understood by analyzing the comparative effects of NAA and fusicoccin on collocyte extension (work in preparation).

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