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Transverse Viscoelastic Extension in *Nitella*

II. EFFECTS OF ACID AND IONS[1]

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ABSTRACT

The transverse viscoelastic extension of isolated *Nitella* cell walls is stimulated by acid pH and by Mg^{2+} and K^+ ions. In the presence of 1 millimolar citrate-phosphate buffer the threshold pH in the transverse direction is 3.5, compared to 4.5 in the longitudinal direction. The relative amounts of extension stimulated by acid are comparable in the two directions at their respective thresholds. Longitudinal and transverse Mg^{2+} ion-induced extensibility begins at 10 millimolar and reaches a plateau between 10 and 100 millimolar. The threshold for K^+ ion enhancement is near 10 millimolar in the longitudinal direction and 50 millimolar in the transverse direction. Maximum stimulation by K^+ is obtained at 250 millimolar. At their respective maxima, Mg^{2+} and K^+ induce equal amounts of extension. However, the relative amount of extension induced by ions is significantly less in the transverse than in the longitudinal direction. Ions and acids appear to affect different sites in the wall, inasmuch as neither treatment abolishes the effect of the other. Walls from rapidly growing cells are more sensitive to stimulation than nongrowing cells in the longitudinal direction but not in the transverse direction.

The longitudinal extensibility of *Nitella* cell walls is increased by acid pH and by certain monovalent and divalent cations (4). Acid-enhanced wall creep is inhibited by other divalent and trivalent cations, including Ca^{2+} and Al^{3+}. There is evidence that protons and cations interact nonenzymically with cell wall carboxyl groups to promote longitudinal creep in *Nitella* (4, 7–9).

Recently we have examined some properties of transverse extensibility in isolated *Nitella* wall loops (5, 6). Mechanical anisotropy, as first reported by Probine and Preston (10), was confirmed in creep studies. The yield point in the transverse direction is about twice that in the longitudinal direction, consistent with the stress ratio for a cylindrical cell (6). Longitudinal extensibility (strain/time/stress) exceeds transverse extensibility by two to seven times, depending on the age and growth rate of the cell. Unlike longitudinal extensibility, transverse extensibility does not correlate significantly with the growth rate.

To characterize lateral wall extension further, we have studied the creep response to acids and ions in the transverse direction. Our results suggest that similar bonds regulate extensibility in the longitudinal and transverse directions. A preliminary account of this work has appeared previously (5).

MATERIALS AND METHODS

The conditions for growing *Nitella* and the method for measuring longitudinal and transverse wall extensibility have already been described (4, 6). The walls were extended in the standard buffer solution using a force calculated from the turgor pressure (2) and the cell size in order to approximate *in vivo* stress. Extension in the standard buffer was measured between 1 and 10 min. Then the solution was drained rapidly from the perfusion chamber (15 s) and replaced by the test solution. The extension in the new medium was measured from 11 to 20 min. When such creep curves are plotted as a function of the logarithm of time they are linear for at least two decades of log time. The data were expressed as the per cent increase in the creep rate, determined from the slopes of extension *versus* log time plots. The per cent strain obtained for walls of growing cells was 2.16 ± 0.51%/l to 10 min ($N = 5$) in the longitudinal direction and 0.52 ± 0.31%/l to 10 min ($N = 7$) in the transverse direction. Growth rates of individual cells were measured over a 24-h period. The standard buffer was 1 mM citrate-phosphate (pH 6.5). Ion solutions were prepared in the standard buffer.

RESULTS

The first detectable stimulation of transverse creep by acid occurred at pH 3.5 (Fig. 1). Acid-stimulated creep was not inhibited by boiling in either methanol or water for 15 min, indicating that a physical rather than an enzymic mechanism is involved. Figure 2 illustrates the relationship between the stimulation obtained with acid and the *in vivo* growth rate. Whereas longitudinal acid-induced creep varied with the growth rate (Fig. 2A), transverse acid-induced creep did not (Fig. 2B). It should be noted that transverse extension has been plotted against longitudinal rather than transverse growth rate. This is valid since Green (1) has shown that there is a constant proportionality (4.5:1) between the relative growth rates in length and diameter. It is possible that the technique of uniaxial extension is not sensitive enough to resolve the smaller differences in acid-enhanced extensibility expected in the transverse direction. The per cent increase in the creep rate due to acid is greater in the transverse than in the longitudinal direction. This may be due to the fact that pH 3.5 was used in the transverse direction while pH 4.5 was used in the longitudinal direction, which correspond to their respective thresholds for acid-induced extension.

From the series of cations known to stimulate longitudinal extension (4) two were selected, K^+ and Mg^{2+}, which at 10 mM caused different amounts of wall loosening. Dose response curves in the longitudinal and transverse directions are shown in Figure 3, A and B. At 10 mM K^+ is less effective than Mg^{2+} in the longitudinal direction, and has no effect on transverse extension. Concentrations below 10 mM were not tested. At higher concentrations K^+ stimulation increases to the level of Mg^{2+}. Ca^{2+} and Al^{3+} ions, which both inhibit acid-induced longitudinal extension (4), also inhibit acid-induced (pH 3.5) transverse extension at 100 mM (Fig. 4).

A plot of ion-induced creep *versus* the endogenous growth rate is shown in Figure 5, A and B. Ion stimulation shows the same correlation with growth rate as acid stimulation in the longitudinal

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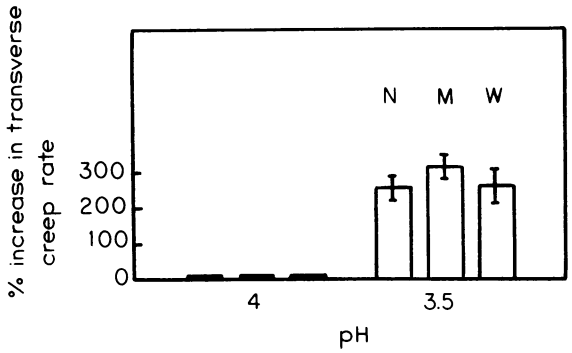

FIG. 1. Stimulatory effect of acid pH on transverse extension of isolated *Nitella* walls. N: nonboiled; M: 15 min methanol-boiled; W: 15 min water-boiled. Vertical bars: SE of the mean, $N = 5$.

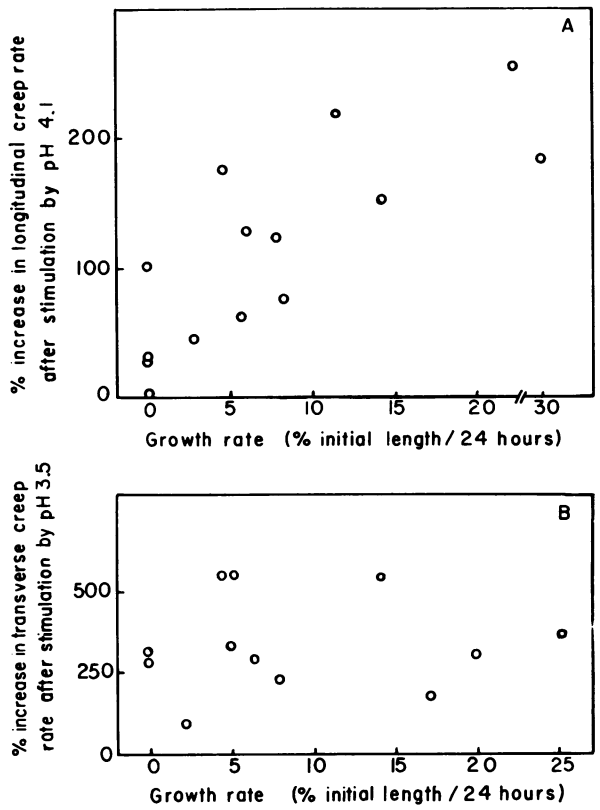

FIG. 2. Acid-stimulation of longitudinal (A) and transverse (B) extension of isolated *Nitella* walls as a function of their growth rates *in vivo*. The force applied was equivalent to the calculated *in vivo* stress due to turgor pressure for each cell. The buffer (1 mM citrate-phosphate) pH was 4.1 in A and 3.5 in B, which are near the threshold for acid stimulation in each of the two directions.

direction (Fig. 5A), and the same lack of correlation in the transverse direction (Fig. 5B).

Since acid and ion stimulation showed the same developmental patterns we tested the possibility that both treatments affected the same sites in the wall. Figure 6 illustrates two reciprocal experiments on the effects of sequential treatments on longitudinal extension, to determine if the stimulation by one could be eliminated by a previous treatment with the other. In curve a, creep was stimulated first with pH 3.5 followed by 10 mM Mg^{2+} at pH 6.5. Acid pretreatment did not eliminate Mg^{2+} stimulation. In the reciprocal treatment (curve b), pH 3.5 treatment gave a further stimulation after prior exposure to 10 mM Mg^{2+}.

DISCUSSION

As previously demonstrated, *Nitella* cell walls can extend viscoelastically in the transverse direction, in spite of the transverse arrangement of the cellulose microfibrils (4). Here we have been able to show that transverse extension is enhanced by acid pH, K^+, and Mg^{2+}, and inhibited by Ca^{2+} and Al^{3+}. The mechanism of acid enhancement, based on boiling experiments, appears to be nonenzymic, and probably involves ion exchange (4, 7–9). In the presence of dilute buffers (1 mM) the threshold for acid enhancement is 4.5 in the longitudinal direction and 3.5 in the transverse direction. We have observed recently that at higher buffer concentrations (10 mM) the pH threshold is shifted upward by ~0.5 pH units, indicating that the wall has a significant buffering capacity (Richmond, Métraux and Taiz, unpublished data). The anisotropy of the pH threshold remains constant.

The results show that acid- and ion-labile bonds are participating in the transverse reinforcement of the wall. Such bonds must be located either within the matrix or at the matrix-cellulose interface. The wall is about 10 times more sensitive to protons (responds at a full pH unit higher) in the longitudinal direction than in the transverse direction. Assuming that the acid-labile sites in the wall are randomly oriented, the transversely arranged microfibrils might simply act as a drag on acid-induced extension (in the same manner as it does on extension at neutral pH) until the matrix has been loosened beyond some critical point. This

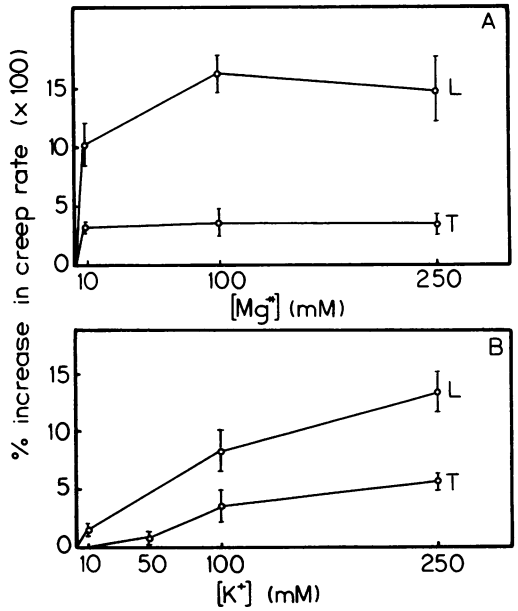

FIG. 3. A: effect of Mg^{2+} concentration on longitudinal (L) and transverse (T) extension of isolated *Nitella* walls. B: effect of K^+ concentration of longitudinal and transverse extensions. Solutions were made up in the standard buffer (pH 6.5). Vertical bars: SE of the mean, $N = 5$ for transverse, $N = 5$ for longitudinal.

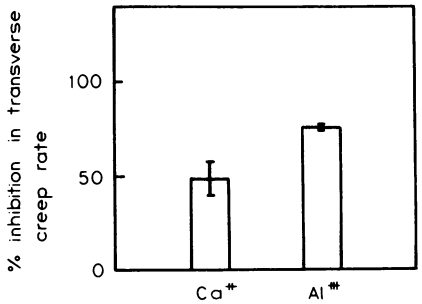

FIG. 4. Inhibitory effects of Ca^{2+} and Al^{3+} on transverse creep after stimulation by pH 3.5. Vertical bars: SE of the mean, $N = 5$. Concentration for both ions was 100 mM.

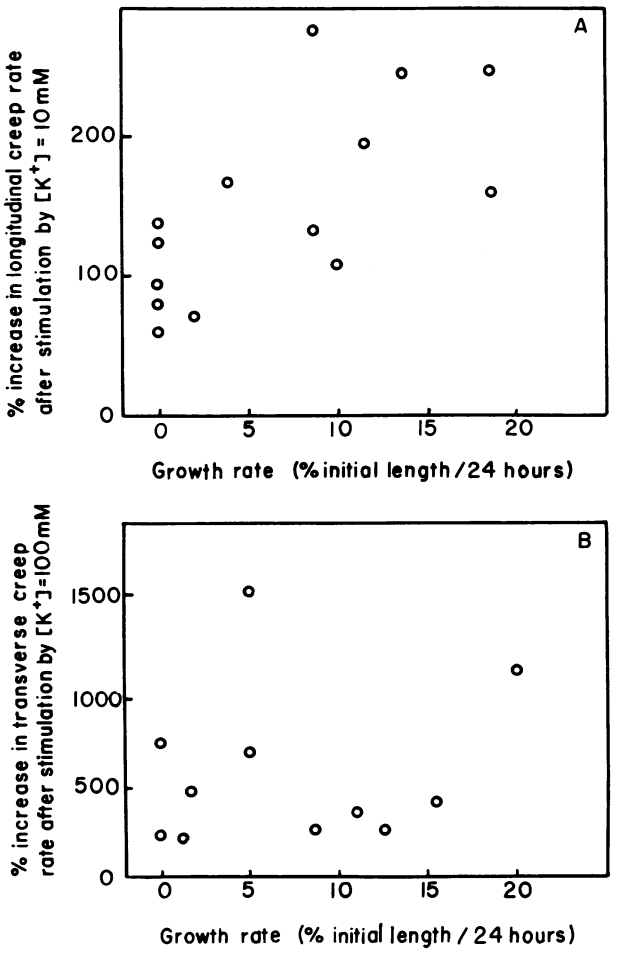

FIG. 5. K^+ stimulation of longitudinal (A) and transverse (B) extension as a function of the *in vivo* growth rate. The applied force was equivalent to the calculated stress due to turgor pressure for each cell. Concentrations were chosen near the threshold value for stimulation in the two directions: 10 mM KCl in A and 100 mM KCl in B. In standard buffer, pH 6.5.

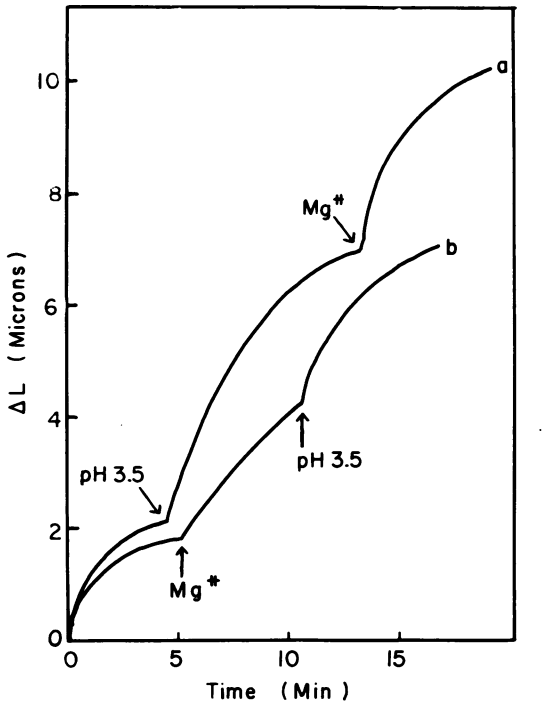

FIG. 6. Effects of sequential treatment with acid pH and Mg^{2+} on longitudinal extension of isolated *Nitella* walls. Curve a = pH 3.5 followed by 10 mM Mg^{2+} pH 6.5. Curve b = 10 mM Mg^{2+}, pH 6.5 followed by pH 3.5. The curves represent two wall strips taken from the same cell.

does not exclude the possibility that a certain degree of anisotropy in the matrix might also reinforce the wall transversely. Morikawa *et al.* (9) have shown that acid-labile carboxyl groups are mainly oriented with their O-O lines perpendicular to the cell axis. This orientation is more pronounced in old cells than in young cells (8), while mechanical anisotropy is greater in young cells than in old cells (6). It seems unlikely that oriented carboxyl groups play a significant role in wall mechanical anisotropy.

The lower pH threshold for acid-enhanced transverse extension suggests that if growth is regulated by wall acidification, longitudinal extension would be preferentially stimulated. The presence of H^+ and OH^- secreting bands along the lengths of *Nitella* internode cells provides an opportunity to test for acid-enhanced growth. Our results (3) showed that elongation is greater in the acidic regions than in the alkaline regions. The pH of the wall in the acid zone is low enough to promote elongation, but not low enough to stimulate transverse extension (Métraux and Taiz, unpublished data). This explains why the cell does not bulge out laterally in the acid zones.

Transverse extension is also stimulated by ions, and the dose response curves are similar to longitudinal extension. At low concentrations Mg^{2+} is more effective than K^+, while at saturating concentrations the stimulations obtained by the two ions are about equal. This implies that the ions act at the same site or equivalent sites, and that the site is more accessible to Mg.

In a previous report we were able to differentiate between acid- and ion-labile wall bonds on the basis of their differing susceptibility to prolonged water boiling. Acid-enhanced creep was inhibited 50 to 75% after 12 h, while ion-enhanced creep was inhibited only 17% (4). In this study it was shown that in sequential treatments with acid pH and Mg^{2+}, neither treatment abolished the effect of the other. This strongly supports the supposition that two separate sites are involved, although it does not rule out a certain amount of overlapping. Morikawa *et al.* (7–9) have also differentiated between acid- and ion-labile sites on the basis of their differing effects on carboxyl group orientation. Protons altered the orientation of the carboxyl groups, while ions did not.

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