The Effect of Sulfhydral Inhibitors on Plant Cell Elongation[1]

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Elongation of plant cells is a metabolic process. It involves a relaxation of wall bonding or wall loosening which is controlled by auxin and thought to require direct participation of enzymes (1, 2, 5, 7, 8). Inhibitors of RNA and protein synthesis stop elongation independently of the degree of wall loosening (2, 5, 7, 8), and N-ethylmaleimide (NEM), a specific sulfhydryl reagent (14), blocks cell elongation without significantly affecting wall loosening. These results with inhibitors suggest that enzymes are not only involved in wall loosening but also catalyze the expansion of the auxin-loosened walls.

Materials and Methods

Sections (1 cm long) cut 0.3 cm below the apical hook (9) from the third internode of eight days old etiolated pea seedlings (*Pisum sativum* var. Alaska) were placed in cold distilled water for 15 to 30 minutes before use. Ten sections per treatment were incubated in 1 x 5 cm petri plates containing 3 to 5 ml of solution. Solutions were buffered with 2.5 mM potassium maleate, pH 4.5. Increase in section length was measured with a stage micrometer fitted to a binocular dissecting microscope.

Extensibility of tissue boiled in methanol and rehydrated in water was determined using an Instron linear stress strain analyzer (10) which provided a direct measure of cell wall loosening. Tissue deformability (a measure of cell wall loosening and turgor pressure) was determined by bending methods (6, 7).

Conductivity of incubating solutions (10 sections per 3 ml) was determined using an Industrial Instruments Model 162B conductivity bridge (Industrial Instruments, Cedar Grove, N. J.) with a Beckmann 3997-P pipet type conductivity cell calibrated against sodium chloride solutions (1 mM NaCl = 100 μmhos).

Results

Elongation of sections treated with auxin (0.1 mM 2,4-D) in the presence of 0.1 to 1 mM NEM seldom exceeded that of untreated controls (Fig. 1A, Table 1). Elongation of sections incubated with 0.1 mM NEM alone was inhibited about 50 percent during the first hour and was completely blocked during the second hour (Fig. 1A). The initial decline in cell wall extensibility of sections incubated without auxin was accelerated by 0.1 mM NEM but after about 2 hours, the extensibilities of control and NEM-treated sections were nearly equal (Fig. 1B). Extensibility of tissues treated with 2,4-D remained high both in the presence and absence of NEM (Fig. 1B, Table 1).

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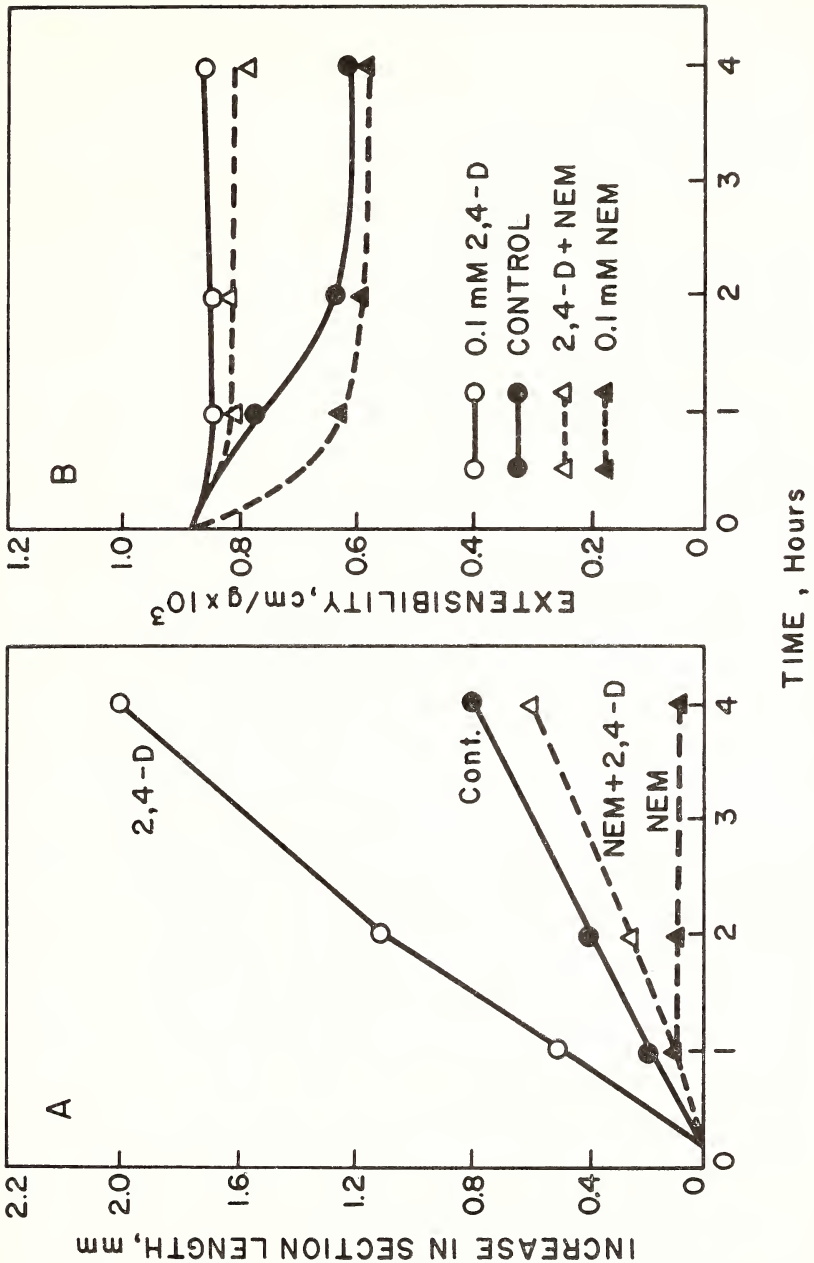

Figure 1. Time course of cell elongation (A) and extensibility (B) of 1 cm etiolated pea 3rd internode sections in the presence and absence of 0.1 mM 2,4-D with and without 0.1 mM N-ethylmaleimide (NEM).

After a 4 hour incubation, sections treated with 2,4-D ± 1 mM NEM were about twice as deformable as untreated controls (Table 2). Both residual and total deformability of the control sections declined whereas that of the 2,4-D-treated sections increased slightly. Deformability with NEM alone (50°) was higher by 20° than that predicted from cell wall extensibility (30°) suggesting a loss of cell turgor as a possible consequence of NEM treatment.

TABLE 1.

Effect of 1 mM N-Ethylmaleimide and Dithiothreitol on Expansion and Cell Wall Extensibility of Pea Internode Tissue in the Presence and Absence of 0.1 mM 2,4-D. Sections were Incubated for 4 Hours.

Treatment	Change in Length After 4 hr (mm)		Extensibility After 4 hr (cm/g × 10^3)	
	—2,4-D	+ 2,4-D	—2,4-D	+ 2,4-D
None	0.7 ± 0.2*	2.7 ± 0.5*	0.48	0.66
N-Ethylmaleimide (NEM)	0.3 ± 0.1	0.8 ± 0.1	0.44	0.64
Dithiothreitol (DTT)	0.6 ± 0.1	2.4 ± 0.4	0.46	0.64
NEM + DTT	1.0 ± 0.3	2.2 ± 0.6	0.52	0.62

* One standard deviation from the mean.

TABLE 2.

Deformability (7) of Pea Internode Tissue Treated With and Without 1 mM N-Ethylmaleimide in the Presence and Absence of 0.1 mM 2,4-D. Sections were Incubated for 4 Hours.

Treatment	Deformabilty, Degrees	
	Total	Residual
Initial (0 Hours)	60 ± 5*	23 ± 2*
Control (4 Hours)	35 ± 5	9 ± 1
2,4-Dichlorophenoxyacetic acid (2,4-D)	77 ± 2	24 ± 2
N-Ethylmaleimide (NEM)	50 ± 7	11 ± 1
NEM + 2,4-D	63 ± 1	18 ± 2

* One standard deviation from the mean.

Conductivity of the incubation medium was used as a measure of NEM effects on electrolyte leakage. Between 1 and 4 hours, the net rate of leakage from NEM-treated sections was about twice that of controls but was unaffected by 2,4-D (Fig. 2A, Fig. 3). To estimate the total change in electrolyte absorption and release, sections were autoclaved for 10 mintes at 14 lb. per sq. inch at 120° C. in 3 ml of media. After cooling, the conductivity of the solution was measured and con-

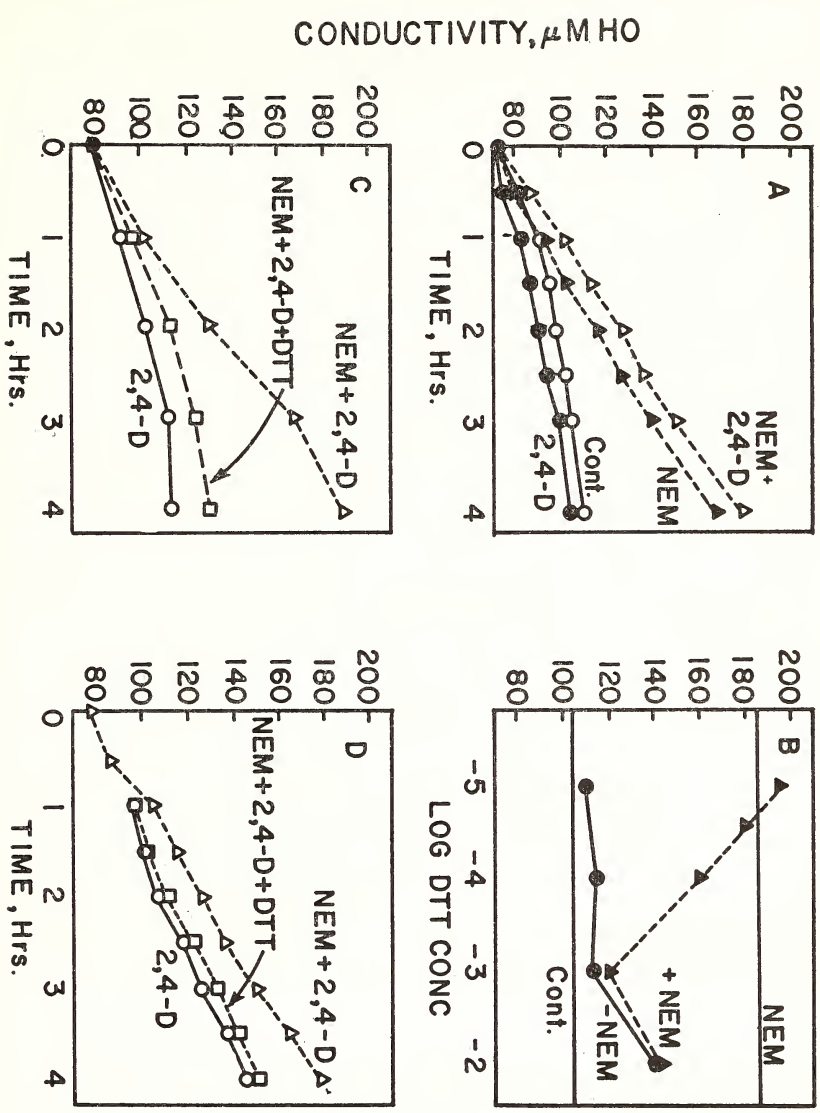

Figure 2. Conductivity of media in which ten 1 cm pea internode sections were incubated in the presence and absence of 1 mM N-ethylmaleimide (NEM) as a function of incubation time ± 0.1 mM 2,4-D (A); as a function of dithiothreitol (DTT) concentration after 4 hrs (B); as a function of incubation time in various combinations with 0.1 mM 2,4-D and 1 mM DTT (C); and transferred to 2,4-D only or 1 mM NEM + 1 mM DTT for 3 hrs after a 1 hr preincubation in 0.1 mM 2,4-D + 1 mM NEM (Tissue treated with NEM + 2,4-D throughout the experiment was not transferred). (D).

ductivity changes during incubation were expressed as percent of autoclaved values corrected for initial conductance due to buffer and added chemicals (Fig. 3). Thus, at the end of 4 hours, the net NEM-induced leakage was equivalent to 20 percent of the total electrolytes.

Dithiothreitol (DTT), a sulfhydryl protectant (4), at a concentration of 1 mM eliminated the NEM-induced leakage and growth inhibition in the absence (Fig. 2B, Table 1) and presence (Fig. 2C, Table 1) of 2,4-D. DTT did not significantly affect either growth in the absence of NEM or wall extensibility measured after 4 hours (Table 1).

Maximum rate of leakage was established by preincubation of tissue in NEM and 2,4-D for 1 hour. When preincubated sections were then transferred to 2,4-D alone or 2,4-D + NEM + DTT for an additional 3 hours, the rate of leakage of the transferred sections was found to be similar to that of sections treated with NEM + 2,4-D throughout the 4 hour period (Fig. 2D). However, a small amount of growth was restored by transfer from NEM + 2,4-D to NEM + 2,4-D + DTT (Table 3).

TABLE 3.

Effect of 1 mM Dithiothreitol on Expansion of Pea Internode Tissue Pretreated with I mM N-Ethylmaleimide + 0.1 mM 2,4-D.

Pretreatment (1 hr)	Treatment (3 hr)	Change in Length After 4 hr (mm)
None	None	1.0 ± 0.2*
2,4-D	2,4-D	2.6 ± 0.2
N-Ethylmaleimide (NEM)	N-Ethylmaleimide (NEM)	0.5 ± 0.2
2,4-D + NEM	2,4-D + NEM	1.2 ± 0.1
2,4-D + NEM	2,4-D	1.3 ± 0.1
2,4-D + NEM	2,4-D + NEM + Dithiothreitol	1.6 ± 0.2

* One standard deviation from the mean.

Discussion

Growth inhibition by sulfhydryl reagents is a well established phenomenon (1, 11, 12, 13) and mercuric chloride is similar to N-ethylmaleimide in its ability to inhibit auxin-induced cell expansion independently of wall loosening (8). High concentrations (1mM) or prolonged incubation with low and intermediate concentrations of NEM may affect membrane semipermeability as evidenced by increased rates of solute leakage. Growth of some tissues are extremely sensitive to changes in external osmotic concentration (8) and Cleland (3) has pointed out that a decrease in the internal osmotic pressure of less than 20 percent will account for a 50 percent reduction in growth rate of Avena coleoptiles.

However, with the information available, it is not possible to equate NEM-induced solute leakage with the cessation of growth. If conductivity changes reflect the loss of osmotically active solutes, the change due to NEM after 1.5 hours (when growth inhibition is complete) accounts for

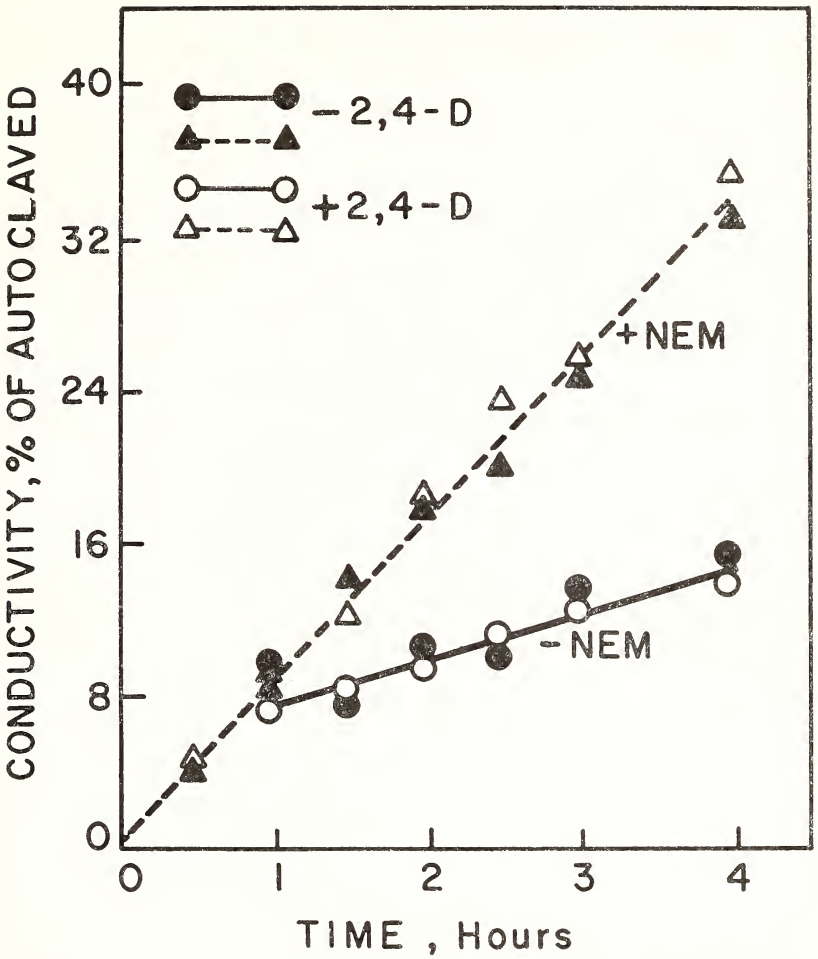

Figure 3. Conductivity of media in which ten 1 cm pea internode sections were incubated in the presence and absence of 0.1 mM 2,4-D with and without 1 mM N-ethylmaleimide (NEM). Values are expressed as percent of autoclaved values corrected for initial conductivity due to buffer and added chemicals.

only a 5 to 6 percent reduction in internal osmotic pressure. Even at 1 mM NEM, solute leakage is not detected during the first hour of NEM treatment and at lower NEM concentrations, this period may be several hours. Yet growth is 50 percent inhibited during the first hour and eliminated thereafter by NEM concentrations as low as 0.1 mM. Thus, the seemingly obvious conclusion that NEM inhibits growth by disruption of the semipermeability properties of the cellular membranes and a concomitant reduction in cell turgor is not readily verified by the data available. Furthermore, when sections were first incubated in NEM and then transferred to a sulfhydryl protectant (DDT) + NEM + 2,4-D,

some growth was restored with no effect on the rapid rate of solute leakage characteristic of prolonged NEM treatment.

Direct measurements of turgor are difficult. An indirect indicator of turgor changes is provided by a comparison of tissue deformability (by bending methods) with wall extensibility (by fiber testing methods). A loss of turgor results in greater deformability than predicted from cell wall extensibility, as shown by sections treated with NEM in the absence of 2,4-D. However, a corresponding loss of turgor is not apparent in sections treated with NEM + 2,4-D even though both sets of sections are equally leaky in conductivity studies.

The somewhat equivocal relationship between NEM effects on growth and turgor do not detract from the observation that auxin-induced wall loosening is not significantly affected by the NEM treatment under conditions where growth is completely inhibited. Although many hormone effects appear to be mediated through nucleic acid metabolism, the results with NEM make it less clear that the auxin effect on wall loosening is under direct nuclear control. The apparent tolerance of the wall loosening machinery to NEM and other sulfhydryl inhibitors is suggestive of a lower order of complexity. An interaction of auxin with a pre-existing protein whose functioning is not appreciably altered by sulfhydryl poisons seems a more reasonable expectation.

Summary

N-ethylmaleimide, a specific sulfhydral inhibitor, blocks cell elongation in etiolated pea third internode sections without significantly affecting wall loosening induced by auxin. Electrolyte leakage induced by high and intermediate N-ethylmaleimide concentrations and the attendant reduction in growth rate appear to proceed independently. The results suggest that the N-ethylmaleimide inhibition of cell expansion is mediated through an inhibition of growth active enzymes and that auxin interacts with a pre-existing protein whose function in cell wall loosening is not appreciably altered by incubation of the tissue with sulfhydryl inhibtors.

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