Lithium Decreases Cold-Induced Microtubule Depolymerization in Mesophyll Cells of Spinach

Michael E. Bartolo and John V. Carter

Departments of Horticultural Science and Plant Biology, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Freezing, dehydration, and supercooling cause microtubules in mesophyll cells of spinach (Spinacia oleracea L. cv Bloomsdale) to depolymerize (ME Bartolo, JV Carter [1991] Plant Physiol 97: 175-181). The objective of this study was to gain insight into the question of whether microtubules depolymerize as a direct response to environmental stresses or as an indirect response to cellular changes that accompany the stresses. Leaf sections of spinach were treated with Li⁺ before and during exposure to low temperature. Treatment with Li⁺ decreased the amount of microtubule depolymerization in cells subjected to low temperature, relative to a nontreated control, raising the possibility that the microtubules in these cells may not be inherently cold labile. Rather, microtubule depolymerization may be in response to cold-induced changes in concentration of cytoplasmic components.

Microtubules, one of the components of the plant cytoskeleton, are involved in several functions critical to plant growth and development, such as mitosis, cell wall formation, determining the plane of cell division, and intracellular transport (8). Microtubules are dynamic structures, existing in an equilibrium between soluble microtubule-protein and the polymerized filament. The major microtubule-protein is tubulin. In animal cells, MAPs make up a significant part of microtubules and are essential for microtubule function. In plants, MAPs have not been unambiguously characterized. Environmental stresses such as freezing, low temperature, and dehydration shift the equilibrium between intact microtubules and dissociated microtubule-protein toward the depolymerized state (2). Several chemical compounds and ions, including Ca²⁺, also cause microtubules to depolymerize.

Most microtubules in cells of warm-blooded animals are inherently cold labile (6), as shown by in vitro studies and by the responses of in vitro-generated microtubules to low temperature. Although the cold stability of microtubules appears to be higher in plants than in animals (7), the knowledge of cold stability of plant microtubules has resulted almost solely from in vivo studies. Difficulties associated with isolating tubulin from plant tissues (12) have kept researchers from determining the cold stability of in vitro-generated plant microtubules that have not been stabilized artificially or separated from putative MAPs. As a result, we do not know whether the effect of low temperature on plant microtubules in vivo is a direct result of a temperature-induced shift in the equilibrium away from the polymerized form or whether their cold lability is due to an interaction of microtubules with another cytoplasmic component, the concentration of which is affected by the decrease in temperature.

Our objective in this study was to learn more about the inherent cold stability of plant microtubules. We attempted to interfere with cold-induced increases in cytoplasmic Ca²⁺ concentration (19). Ca²⁺ was singled out because most microtubules are destabilized by it at very low concentrations and because in animal cells changes in Ca²⁺ concentration can be a part of the transduction of environmental signals (4). Although signal transduction is not as well understood in plants as in animals, several potential intermediates in the phosphoinositide transduction pathway have been identified in plants (15), and a similar mechanism has been suggested to exist in plant cells (13). In animal systems, Li⁺ inhibits inositol polyphosphate 1-phosphatase (9) and inositol monophosphate phosphatases (1), enzymes involved in the turnover of polyphosphoinositides, thus inhibiting Ca²⁺ release from intracellular pools (4). To block cold-induced increases in cytoplasmic Ca²⁺ concentration, we applied Li⁺ to spinach leaf tissue. Here we report the response to microtubules in tissues treated with Li⁺ and then cooled.

MATERIALS AND METHODS

Plant Material

Four-week-old spinach plants (Spinacia oleracea L. cv Bloomsdale) were grown from seed in a controlled environment growth chamber as previously described (2). Leaves measuring approximately 5 cm in length from base to tip were used for all experiments.

Li⁺ Treatment

The lower cuticle and epidermis were manually stripped from leaves, and 4-mm² sections were excised from the peeled area. The tissue pieces were then placed peeled-side-down on solutions of 25 or 50 mM LiCl or on a distilled water...
LI⁺ DECREASES COLD-INDUCED MICROTUBULE DEPOLYMERIZATION

Figure 1. The index of microtubule polymerization in mesophyll cells of spinach that were treated with water (control) or 50 mM Li⁺ and cooled to low temperatures without ice formation. RT, Room temperature. Within each treatment, at least 100 cells, chosen at random, were observed. The fraction of the cells containing full arrays, partial arrays, or no microtubules was determined. Cells with full arrays were counted as "1," with partial arrays as "0.5," and cells with no microtubules were scored as "0." The total of these values divided by the number of cells counted was the initial index of polymerization. Pairwise comparisons between treatments, made to take into account the variation in the content of intact microtubules in cells having partial arrays, contributed no more than 5% to the final value of the index of polymerization. The identity of the treatments was not known by the microscopist until data from all treatments had been recorded. For examples of spinach mesophyll cells with fully intact and partial microtubule arrays, as well as a cell with completely depolymerized microtubules, see figure 2 of ref. 2.

control for 1 h. As a control for a general univalent cation effect, leaf pieces were incubated on 25 mM NaCl. To see whether the effect of Li⁺ could be negated, leaf pieces were incubated on a solution containing 25 mM LiCl and 50 mM myo-inositol.

Low-Temperature Treatment and Supercooling

After treatment, leaf sections were placed peeled-side-down on one to two drops of 25 or 50 mM LiCl distilled water, 25 mM NaCl, or 25 mM LiCl + 50 mM myo-inositol in 10-mL flat-bottomed vials. The leaf sections were held at 2°C for 1 h and then cooled 1°C every 30 min to a final temperature of −6°C. At each test temperature, the samples were held an additional 30 min during which time one sample was fixed for indirect immunofluorescence. All vials were closely inspected to ensure that freezing did not occur.

Indirect Immunofluorescence Microscopy

The presence of intact microtubule arrays was assessed via indirect immunofluorescence microscopy with anti-tubulin antibodies based on the procedure of Wick and Duniec (18) as modified by Bartolo and Carter (2). The quantitation of microtubule polymerization status (index of polymerization) was based on a system described earlier (2).

RESULTS AND DISCUSSION

Depolymerization of plant microtubules during exposure to low temperature is thought to be an inherent property of microtubules. Some of our previous results, however, suggested that depolymerization of microtubules in spinach mesophyll cells on exposure to low temperature, dehydration, and freezing may be mediated by another cytoplasmic component and not a direct effect of the stresses on microtubule stability (2).

One way to test the inherent cold lability of microtubules is to prevent other low temperature-induced changes in the cytoplasm, thus preventing temperature-induced alterations in interactions of microtubules with other cytoplasmic components. Preventing all temperature-induced changes other than cytoskeletal ones is obviously not possible, therefore, we narrowed our focus to changes related to Ca²⁺. We used Li⁺ because it blocks increases in Ca²⁺ in plant cell protoplasm induced by low temperature (19) and by salinity stress (11).

Pretreating spinach leaves with Li⁺ greatly decreased the amount of microtubule depolymerization in mesophyll cells subjected to low temperatures (Fig. 1). This phenomenon was seen with both 50 mM (Fig. 1) and 25 mM (Fig. 2) Li⁺. More intact microtubules were present in cells treated with 50 mM Li⁺ than in those treated with 25 mM Li⁺. This stabilization was not a general salt effect, because it was not produced by treatment with Na⁺. Rather, Na⁺ initiated slightly more microtubule depolymerization than occurred in an untreated control (compare Figs. 1 and 2).

Li⁺ (25 mM) also decreased microtubule depolymerization when cells were subjected to freezing stress (data not shown). Despite some loss of cellular compartmentalization due to freezing injury, which may have resulted in release of Ca²⁺ into the cytoplasm, it was still possible to discern the Li⁺ effect. Because supercooling of spinach mesophyll cells causes no such loss of compartmentalization (3), it, rather than freezing, was used as the microtubule-destabilizing treatment in this study.

In addition to its effect on the phosphoinositol pathway, several other effects of Li⁺ could be responsible for our observations. Two alternative hypotheses than cannot be ruled out by our results are: (a) Li⁺-induced ethylene may enhance microtubule cold stability (16, 17). We did not observe any change in the orientation of microtubules in

Figure 2. The index of microtubule polymerization in mesophyll cells of spinach that were treated with 25 mM NaCl, 25 mM LiCl, or 25 mM LiCl plus 50 mM myo-inositol and cooled to low temperatures without ice formation. RT, Room temperature. See legend for Figure 1 for a description of the method for obtaining the index of polymerization.
spinach mesophyll cells, as would be expected if our results were due to a Li⁺-induced increase in ethylene levels (16). Cortical arrays of microtubules are not as highly ordered in spinach mesophyll cells as in outer cortical and epidermal cells in pea epicotyl and mung bean hypocotyl (16), however. Therefore, our observations do not provide a definitive test of this hypothesis. (b) Li⁺ may stabilize microtubules against cold-induced depolymerization by stabilizing microfilaments (5, 10, 14).

Although these hypotheses cannot now be ruled out, it is also clear that Li⁺ does affect cytoplasmic Ca²⁺ levels in plants. Li⁺ inhibits the salt-induced release of Ca²⁺ from stored pools in maize protoplasts, and this inhibition is negated by added inositol (11).

We have found that pretreating spinach leaves with 25 and 50 mM Li⁺ decreases cold-induced microtubule depolymerization. When inositol was added along with Li⁺, the Li⁺ effect on microtubule cold stability was not affected at 0 and −2°C, but it was diminished at −4 and −6°C (Fig. 2). It is possible that inositol does not enter the cytoplasm of these walled cells or that entry is slowed by the walls or plasmalemma such that equilibration between intra- and extracellular concentrations requires several hours. Experiments are now underway to establish whether Li⁺-induced microtubule cold stability is due to an inhibition by Li⁺ of the cold-induced increase of cytoplasmic Ca²⁺ concentration or to the other possible ways that Li⁺ could affect microtubules.

LITERATURE CITED