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Quantitation of Chill-Induced Release of a Tubulin-Like Factor and Its Prevention by Abscisic Acid in *Gossypium hirsutum* L.[1]

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ABSTRACT

The degree of tubulin polymerization in cotton (*Gossypium hirsutum* L. cv Acala) cotyledonary tissue was estimated by radioimmunoassay which measured the amount of a tubulin-like factor. It was assumed that the release of this tubulin-like factor indicated depolymerization of microtubules. Exposure to chilling resulted in complete release of the tubulin-like factor. Pretreatment with abscisic acid in the light almost completely prevented the chill-induced release of the tubulin-like factor. Addition of colchicine during the chilling period accelerated the release of the tubulin-like factor. Pretreatment with abscisic acid greatly reduced this effect of colchicine. It is concluded that the destruction of the microtubular network is involved in the development of chilling injury in cotton. Abscisic acid apparently decreased chilling injury by stabilization of the microtubular network.

Most of the microtubules are cold sensitive and both microtubules of the mitotic apparatus and cytoplasmic microtubules are depolymerized by low temperatures. This was found in animal cells (12), higher plant cells (5–7), algae (8), and protozoa (21). We proposed that chill-induced depolymerization of the microtubular network could be a primary cause of chilling injury. This assumption was based on the fact that microtubule-disrupting agents accelerated and enhanced chilling injury (15). In the present study, we attempted to obtain more direct evidence for the role of microtubules in development of chilling injury by measuring the degree of tubulin polymerization. The degree of tubulin polymerization in the tissue was estimated by a recently developed radioimmunoassay which measured the amount of a tubulin-like factor (16).

MATERIALS AND METHODS

Cotton seeds (*Gossypium hirsutum* L. cv Acala, obtained from Hazera Seed Co., Israel) were germinated and grown for 12 d in a growth chamber. The plants were kept in continuous light (950 ft-c) at 27°C in plastic boxes filled with a mixture of peat and soil (1:1, v/v) and were irrigated with water.

ABA was applied by floating 30 to 40 cotyledonary discs (17 mm in diameter) on 100 ml of the aqueous hormone solution in a Petri dish (14 cm in diameter) under continuous light (950 ft-c) or in darkness at 27°C.

Chilling treatment was given at 4°C in the dark. Groups of two to three discs on 10 ml of distilled H_2O in covered Petri dishes (5.5 cm in diameter) were exposed to chilling. Combined treatment of chilling and colchicine were given by exposing the discs to chilling while they floated on aqueous solution of colchicine.

The amount of free tubulin in the cells was estimated at the end of the exposure to chilling. The tissue was extracted by glass to glass homogenizer in 0.1 M Pipes (piperazine-*N*,*N*′-bis[2-ethane sulfonic acid]disodium salt, pH 6.8) containing 0.1% Triton X-100, 0.1% gelatin, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, 0.1 M 2-mercaptoethanol (0.5 g tissue:2 ml extraction buffer). The homogenate was immediately centrifuged at 100,000*g* for 45 min. The amount of the tubulin-like factor in the supernatant was measured by radioimmunoassay and expressed as μg-eq of calf brain tubulin as earlier described by Rikin *et al.* (16). The total amount of tubulin-like factor in the tissue was determined by the radioimmunoassay after extraction under the previous conditions, but the extraction medium contained 0.5 M guanidine-HCl, and the homogenate was kept at room temperature for 30 min. After centrifugation, the supernatant was dialyzed against the extraction medium without guanidine-HCl. This procedure is assumed to result in complete release of all the tubulin-like factor (16). The amount of free tubulin-like factor is expressed as percentage of the total amount of tubulin-like factor. The total amount of tubulin-like factor in the various experiments was between 850 and 980 μg-eq tubulin/g fresh weight. The results of each table are the average of, at least, three experiments ± SE.

RESULTS

Exposure of cotton cotyledonary tissue to chilling (4°C for 3 d) resulted in almost complete release of the tubulin-like factor so that all the cellular tubulin was found as free tubulin (Table I). Pretreatment with ABA in the light greatly reduced chilling injury (14, 15, 18). Such pretreatment almost completely prevented the chill-induced release of the tubulin-like factor. In chilled tissue that had been pretreated with ABA in the light, the amount of the free tubulin-like factor was almost similar to that found in nonchilled tissue (Table I). The same pretreatment with ABA, but in darkness, was found to be much less effective in reduction of chilling injury (18) and it did not decrease the chill-induced release of the tubulin-like factor (Table II).

Addition of colchicine during the chilling period greatly accelerated the release of the tubulin-like factor as compared to chilled tissue without colchicine (Table III). Pretreatment with ABA decreased the effect of colchicine (Table III).

Treatments with light, darkness, ABA, and colchicine had no effect on the level of the free tubulin-like factor in nonchilled tissue.

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Table I. *Effect of Chilling and Pretreatment with ABA in the Light on the Amount of Free Tubulin-Like Factor in Cotton Cotyledonary Tissue*

Tissue discs were floated on 10 μM ABA solution for 24 h in the light, then exposed to chilling floating on distilled H_2O. The amount of the free tubulin-like factor was determined before and after the chilling period.

Chilling 4°C/3 d	ABA	Free Tubulin-Like Factor
		% of total
−	−	24 ± 2
−	+	24 ± 2
+	−	92 ± 11
+	+	31 ± 3

Table II. *Effect of Pretreatment with ABA in Light or in Darkness on the Amount of Free Tubulin-Like Factor in Cotton Cotyledonary Tissue after 3 Days of Chilling at 4°C*

Tissue discs were floated on 10 μM ABA solution for 24 h in the light (950 ft-c) or in darkness, then exposed to chilling floating on distilled H_2O. The amount of the free tubulin-like factor was determined after the chilling period.

Pretreatment		Free Tubulin-Like Factor
Light	ABA	
		% of total
+	−	89 ± 8
+	+	29 ± 3
−	−	100 ± 3
−	+	97 ± 9

Table III. *Effect of Colchicine and Pretreatment with ABA on the Amount of Free Tubulin-Like Factor in Cotton Cotyledonary Tissue after 1.5 Days of Chilling at 4°C*

Tissue discs were floated on 10 μM ABA solution for 24 h in the light, then exposed to chilling floating on solution of 0.5% colchicine. The amount of the free tubulin-like factor was determined after the chilling period.

ABA	Colchicine	Free Tubulin-Like Factor
		% of total
−	−	38 ± 3
+	−	34 ± 3
−	+	100 ± 2
+	+	64 ± 7

DISCUSSION

We (15) have suggested that chill-induced depolymerization of microtubules is involved in chilling injury. The depolymerization is generally reversible but prolonged shifts towards depolymerization may cause irreversible damage which leads to cell death. This assumption was based on the fact that antimicrotubular drugs such as colchicine, demecolcine, and podophyllotoxin accelerated and enhanced the chilling injury (15). In the present study, it was shown that low temperature which brought about chilling injury in cotton cotyledons (14, 15, 18) caused complete release of the tubulin-like factor. The amount of the tubulin-like factor was measured by a radioimmunoassay (16). It is assumed that the release of the tubulin-like factor may indicate depolymerization of microtubules. However, other causes for the increase in the amount of the tubulin-like factor, such as release from membranes and organelles, should be considered. Similarly to our finding, Ilker *et al.* (5) found by electron microscope that, in chilling-sensitive cotyledons of tomato, one of the first ultrastructural changes was the disappearance of the microtubular network.

Microtubules show different levels of sensitivity to low temperature (1–3, 21), but the basis for these differences is unknown. We found that pretreatment with ABA in the light which decreased chilling injury (14, 15, 17–20), also prevented the chill-induced depolymerization of microtubules as indicated by the prevention of the release of the tubulin-like factor. A normal level of GSH was shown to be essential for maintaining the microtubular network in many kinds of cells. A decrease in the level of GSH was associated with depolymerization of microtubules (9–11, 13). The importance of normal level of GSH for the protection against chilling is supported by the finding that in spruce needles the level of GSH and the activity of GSH reductase are increased during the acclimation period (4). We found that chilling injury in cotton cotyledonary tissue was preceded by a decrease in the level of GSH, while pretreatment with ABA preserved a normal level of GSH (14).

It is suggested that one of the primary causes of chilling injury is the destruction of microtubules. ABA may induce resistance to chilling by stabilization of the microtubular network.

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