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Response of Cotton Mitochondria to Chilling Temperatures[1]

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ABSTRACT

Chilling cotton (*Gossypium hirsutum* L.) seedlings at 5 C for 2 and 4 days reduced the specific activity of succinate oxidation by the isolated root mitochondria. Chill-hardening at 15 C for 2 days prior to the chilling treatment only partially protected against the decrease. When mitochondria were isolated from unchilled plants and activity measured from 5 to 30 C, a break in the activity curve was noted at 15 C. Mitochondrial enzyme inactivations may help explain the nature of chilling injury in cotton.

Additional index words: Chill-hardening, Enzyme inactivation, Arrhenius plot.

SEVERAL reports suggest that mitochondria are involved in chilling injury. The activity of mitochondria from sweet potato (*Ipomoea batatas* Lam.) root declined sharply after the roots were stored 5 weeks at 7.5 C (8). When the roots were stored at 0 C, the oxidative activity declined in only 10 days (13). Richardson and Tappel (15) showed that mitochondria from a cold-blooded animal have the ability to swell rapidly at temperatures down to 0 C, but that particles isolated from a warm-blooded animal could not swell rapidly at low temperature. Similar experiments (11) with chilling-sensitive and chilling-resistant plants demonstrated that mitochondria from chilling-resistant plants were more flexible (as measured by their greater ability to swell) than were mitochondria from chilling-sensitive plants. Recently, Lyons and Raison (10) showed that there was a marked depression in the respiratory rates below 10 C of mitochondria from chilling-sensitive plants. The respiratory depression was not observed with mitochondria from chilling-resistant plants.

In a previous paper (17) we reported that ATP in young cotton (*Gossypium hirsutum* L., cv. Parrott) (a chilling-sensitive plant) was markedly decreased by

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chilling the plant at 5 C. Christiansen and Moore (3) determined that the minimum temperature for hydrolysis of cotton lipid *in vivo* was approximately 15 C. Also, cotton tissues appear to suffer a loss of differential permeability at chilling temperatures (2, 4). These results suggest that mitochondrial and other membrane-related functions are impaired by chilling temperatures in cotton.

The objectives of the work reported here are (a) to determine the effects of *in situ* chilling on the activity of subsequently isolated mitochondria and (b) to determine the *in vitro* response of isolated mitochondria to chilling temperatures.

MATERIALS AND METHODS

Mitochondria were isolated from 3-week-old cotton root tissue. The plants were grown in a fiberglass house or a climate-control chamber under culture conditions previously described (17). One group of plants was chilled at 5 C for 2 and 4 days, while another group was chill-hardened for 2 days (14-hour photoperiod) at 15 C, then subjected to the chilling treatment. The rest were maintained at 30 C day-20 C night as controls. Lateral roots were stripped from the main tap root and washed with distilled water. Twelve grams fresh weight of lateral root tissue were cut into fine pieces in four volumes of cold extracting medium containing 0.4 *M* sucrose, 0.02 *M* N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 0.01 *M* Na_2EDTA, 0.02 *M* citrate, 2% (w/v) bovine serum albumin (18), and 0.05 *M* sodium meta-bisulfite (1). The pH of the medium was adjusted to 7.5 with KOH. Subsequent operations were performed near 0 C. The roots were blended in a Sorvall Omni-Mixer[4] at full speed for 5 seconds; then the homogenate was strained through two layers of fine mesh nylon hose to remove the pulp. The mitochondria were obtained by differential centrifugation (18), washed once with the extracting medium and a second time with a medium containing 0.4 *M* sucrose, 0.02 *M* HEPES, 0.01 *M* KH_2PO_4 and 0.01 *M* KCl, pH 7.2. The final pellet was suspended in 2 to 4 ml of a medium consisting of 0.25 *M* sucrose, 0.312 *M* KH_2PO_4, and 6.25 m*M* $MgSO_4$ at pH 7.2. One ml of this mixture was used as the mitochondria preparation.

Mitochondrial activity was measured as O_2 consumption at 30 C monitored with an oxygen electrode. The total reaction mixture of 2.5 ml at pH 7.2 contained, in micromoles; sucrose, 625; glucose, 125; KH_2PO_4, 125; $MgSO_4$, 2.5; ADP, 2.5; NAD, 2.5; NADP, 0.25; $MnCl_2$, 0.25; cocarboxylase, 0.25; coenzyme A, 0.25; substrate, 125; hexokinase, 0.5 mg; cytochrome C, 1 mg; mitochondria, 0.2 to 0.4 mg protein N. Protein was determined by the method of Lowry et al. (9).

For *in vitro* studies plants were grown at 30 C day-20 C night for 3 weeks, and then the mitochondria isolated as described above. Mitochondrial succinate oxidation was measured with the O_2 electrode from 5 to 30 C in increments of 5 degrees. Activity was first measured at the desired temperature increment, then the reaction mixture was warmed (3 to 7 min) to 30 C. The activity at 30 C was then determined on the same mitochondria used at the lower temperature. A different mitochondrial preparation was used for each 5-degree increment; but by expressing the results as percent of the 30 C rate, determinations were directly comparable. Two preparations were used for each increment.

RESULTS AND DISCUSSION

The total yield of mitochondrial protein per unit fresh weight of tissue was slightly less (10 to 14%) from the chilled plants than from the control plants. However, the effect of chilling was greatest on the specific activity of the isolated mitochondria (Table 1). Chilling the cotton seedling for 4 days at 5 C reduced by one-half the specific activity of succinate oxidation by mitochondria subsequently isolated from the roots. In a similar experiment in which malate was used as substrate, the $QO_2(N)$ was 506 for mitochondria isolated from control roots, compared to 55 for mitochondria from roots chilled for 4 days. We have also observed (unpublished data) that chilling etiolated cotton hypocotyls for 2 days reduces malate oxidation more than α-ketoglutarate oxidation by isolated mitochondria. These results suggest that certain of the tricarboxylic acid cycle enzymes may be more sensitive than others to chilling injury. Inactivation of individual mitochondrial enzymes by low nonfreezing temperatures has been reported (14, 16) for enzymes isolated from animal tissue. Also, inactivation of the phosphorylating system of spinach (*Spinacia aleracea*) mitochondria by freezing was reported by Heber and Santarius (6). They suggested that alternation of lipoprotein was a main cause of injury. Similar inactivations may occur in chilling-sensitive plants at low nonfreezing temperatures.

Table 1. Succinate oxidation rates of mitochondria isolated from unhardened and hardened cotton seedling roots chilled at 5 C.

Temp. regime	Unhardened		Hardened	
	$Q_{O_2}(N)$*	% Control†	$Q_{O_2}(N)$	% Control
15 C for 2 days			985±11	107
5 C for 2 days	650±33	81	623±8	78
5 C for 4 days	491±13	53	676±5	72

* Microliters O_2 hr^{-1} mg N^{-1}. † Control mitochondria were isolated from plants maintained at 30 C day - 20 C night.

After chill-hardening of cotton seedlings at 15 C for 2 days the specific activity of succinate oxidation of the isolated root mitochondria was slightly above the control level (Table 1). Two days of chilling reduced the specific activity by 22%, and an additional 2 days chilling reduced the activity to 72% of the control. The hardening process provides incomplete protection against the chilling injury to cotton mitochondria, although the results after 4 days of chilling suggest that some protection is afforded. Hardening cotton against low temperature injury is a relative condition, since hardened plants are resistant to short periods (4 to 5 days) of chilling but usually succumb to longer periods of low temperature. This susceptibility to prolonged chilling may come about by gradual inactivation of the mitochondrial enzymes.

To determine the direct response of cotton mitochondria to low temperature, particles were isolated from unchilled root tissue and relative succinate oxidation rates determined from 5 to 30 C. An Arrhenius plot of the data is given in Fig. 1. The result is a two-phase linear response to temeprature with the transition point occurring at about 15 C. Except that the transition point is at a slightly higher temperature, the Arrhenius plot is similar to those reported by Lyons and Raison (10) for mitochondria from other chilling-sensitive plants. They suggested that the membrane lipids of chilling-sensitive mitochondria undergo a change in physical state at a certain critical temperature. Below this temperature oxidative activity is greatly depressed.

The transition temperature of 15 C in cotton mitochondria may be significant in light of other observations. First, the minimum temperature for *in vivo* hydrolysis of cotton lipid is near 15 C (3). Since

[4] Mention of a trademark of proprietary product does not constitute a guarantee or warranty of the product by the US Dept. of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

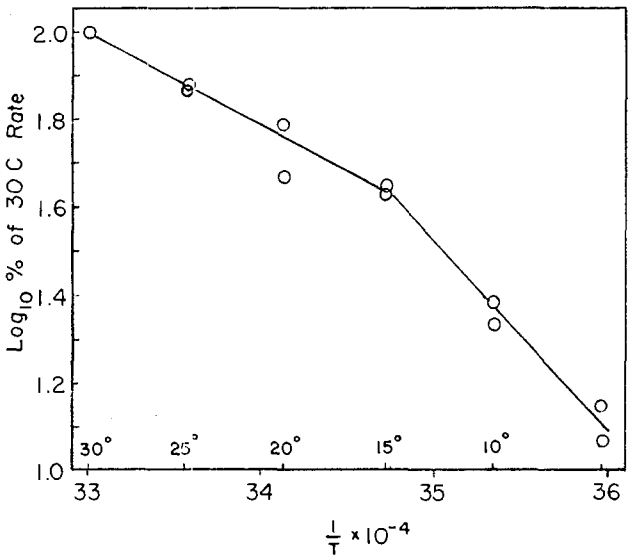

Fig. 1. Arrhenius plot of succinate oxidation by mitochondria isolated from 3-week-old cotton roots. Control represents rate of activity at 30 C. Two preparations were used for each temperature.

lipid metabolism is associated with mitochondria (7) or similar membrane-bound particles, a change in the physical state of the membranes could account for this observation. Second, the optimum temperature for the hardening process appears to be around 15 C. In unhardened cotton plants injury by chilling usually occurs at temepratures lower than the hardening temperature. Apparently the metabolic processes functioning near the transition temperature alter the internal composition of the tissue to the extent that it is less susceptible to normally lethal chilling temperatures. Sugars may be responsible for the protection afforded by hardening, since high levels of sugar protect against mitochondrial enzyme inactivation by low temperature in some cases (6, 16). Guinn (5) showed that sugars accumulated in cotton seedlings during the hardening process.

The *in vivo* loss of specific activity of cotton root mitochondria could result from several factors. With loss of differential permeability at low temperature (2, 4) segregation of cellular functions would be lost and hydrolytic enzymes, released. Release of phenolic materials would also be detrimental. Meyers and Throneberry (12) showed that gossypol, a cotton phenolic, is inhibitory to some oxidative respiratory enzymes and to phosphorylation. Another possibility is that reduced activity of mitochondria may be due to enzyme inactivation directly by the low temperatures (14, 16). This possibility exists for both *in vivo* and *in vitro* response of mitochondria to low temperature. However, the response of isolated cotton mitochondria to chilling temperatures is probably due to the lipid phase transition suggested by Lyons and coworkers (10, 11). Considerable data on chilling injury and hardening are becoming available, but much more are needed before definite conclusions concerning the biochemical causes of the phenomena can be drawn.

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